PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/705, 16/28, G01N 33/566, C07K 14/435, C12Q 1/68, C12N 5/10

A2

(11) International Publication Number: **WO 97/45541**

(43) International Publication Date: 4 December 1997 (04.12.97)

(21) International Application Number: PCT/US97/09553

(22) International Filing Date: 2 June 1997 (02.06.97)

(30) Priority Data:
08/656,055 31 May 1996 (31.05.96) US

(71) Applicants: THE LELAND S. STANFORD JUNIOR UNIVERSITY [US/US]; Office of Technology Licensing, Suite 350, 900 Welch Road, Palo Alto, CA 94304 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US).

(72) Inventors: SCOTT, Matthew, P.; 914 Wing Place, Stanford, CA 94035 (US). GOODRICH, Lisa, V.; 66 Newell Road, Palo Alto, CA 94303 (US). JOHNSON, Ronald, L.; Apartment 7, 1528 Hudson Street, Redwood City, CA 94061 (US). EPSTEIN, Ervin, Jr.; 553A Miner Road, Orinda, CA 94563 (US). ORO, Anthony; 1120 Welch Road #216, Palo Alto, CA 94304 (US).

(74) Agents: ARNOLD, Beth, E. et al.; Foley, Hoag & Eliot LLP, One Post Office Square, Boston, MA 02109 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: *PATCHED* GENES AND THEIR USES

(57) Abstract

Methods for isolating patched genes, particularly mammalian patched genes, including the mouse and human *patched* genes, as well as invertebrate patched genes and sequences, are provided. Decreased expression of *patched* is associated with the occurrence of human cancers, particularly basal cell carcinomas of the skin. The cancers may be familial, having as a component of risk an inherited genetic predisposition, or may be sporadic. The *patched and hedgehog* genes are useful in creating transgenic animal models for these human cancers. The *patched* nucleic acid compositions find use in identifying homologous or related proteins and the DNA sequences encoding such proteins; in producing compositions that modulate the expression or function of the protein; and in studying associated 15 physiological pathways. In addition, modulation of the gene activity *in vivo* is used for prophylactic and therapeutic purposes, such as treatment of cancer, identification of cell type based on expression, and the like. The DNA is further used as a diagnostic for a genetic predisposition to cancer, and to identify specific cancers having mutations in this gene.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PATCHED GENES AND THEIR USES

This invention was made with support from the Howard Hughes Medical Institute. The Government may have certain rights in this invention.

INTRODUCTION

Technical Field

The field of this invention is segment polarity genes and their uses.

Background

Segment polarity genes were originally discovered as mutations in flies that change the pattern of body segment structures. Mutations in these genes cause animals to develop changed patterns on the surfaces of body segments; the changes affecting the pattern along the head to tail axis. Among the genes in this class are *hedgehog*, which encodes a secreted protein (HH), and *patched*, which encodes a protein structurally similar to transporter proteins, having twelve transmembrane domains (*ptc*), with two conserved glycosylation signals.

The *hedgehog* gene of flies has at least three vertebrate relatives- *Sonic hedgehog (Shh); Indian hedgehog (Ihh), and Desert hedgehog (Dhh).* *Shh* is expressed in a group of cells, at the posterior of each developing limb bud, that have an important role in signaling polarity to the developing limb. The *Shh* protein product, SHH, is a critical trigger of posterior limb development, and is also involved in polarizing the neural tube and somites along the dorsal ventral axis. Based on genetic experiments in flies, *patched* and *hedgehog* have antagonistic effects in development. The *patched* gene product, *ptc*, is widely expressed in fetal and adult tissues, and plays an important role in regulation of development. *Ptc* downregulates

transcription of itself, members of the transforming growth factor β and *Wnt* gene families, and possibly other genes. Among other activities, HH upregulates expression of *patched* and other genes that are negatively regulated by *patched*.

It is of interest that many genes involved in the regulation of growth and control of cellular signaling are also involved in oncogenesis. Such genes may be oncogenes, which are typically upregulated in tumor cells, or tumor suppressor genes, which are down-regulated or absent in tumor cells. Malignancies may arise when a tumor suppressor is lost and/or an oncogene is inappropriately activated. Familial predisposition to cancer may occur when there is a mutation, such as loss of an allele encoding a suppressor gene, present in the germline DNA of an individual.

The most common form of cancer in the United States is basal cell carcinoma of the skin. While sporadic cases are very common, there are also familial syndromes, such as the basal cell nevus syndrome (BCNS). The familial syndrome has many features indicative of abnormal embryonic development, indicating that the mutated gene also plays an important role in development of the embryo. A loss of heterozygosity of chromosome 9q alleles in both familial and sporadic carcinomas suggests that a tumor suppressor gene is present in this region. The high incidence of skin cancer makes the identification of this putative tumor suppressor gene of great interest for diagnosis, therapy, and drug screening.

Relevant Literature

Descriptions of *patched*, by itself or its role with *hedgehog* may be found in Hooper and Scott (1989) Cell 59-.751-765; and Nakano *et al.* (1989) Nature 341 -.508-513. Both of these references also describe the sequence for *Drosophila patched*. Discussions of the role of *hedgehog* include Riddle *et al.* (1993) Cell 75-.1401-1416-, Echelard *et al.* (1993) Cell 75:1417-1430- Krauss *et al.* (1993) Cell 75:1431-1444 (1993); Tabata and Kornberg (1994) 76:89-102;

Heemskerk and DiNardo (1994) Cell 76:449-460; and Roelink *et al.* (1994) Cell 76:-761-775.

Mapping of deleted regions on chromosome 9 in skin cancers is described in Habuchi *et al.* (1995) Oncogene 11: 1 671-1674, Quinn *et al.* (1 994) Genes Chromosome Cancer 11:222-225; Quinn *et al.* (1994) J. Invest. Dermatol. 102:300-303; and Wicking *et al.* (1994) Genomics 22:505-51 1.

Gorlin (1987) Medicine 66:98-113 reviews nevoid basal cell carcinoma syndrome. The syndrome shows autosomal dominant inheritance with probably complete penetrance. About 60% of the cases represent new mutations. Developmental abnormalities found with this syndrome include rib and craniofacial abnormalities, polydactyly, syndactyly and spina bifida. Tumors found with the syndrome include basal cell carcinomas, fibromas of the ovaries and heart, cysts of the skin, jaws and mesentery, meningiomas and medulloblastomas.

SUMMARY OF THE INVENTION

Isolated nucleotide compositions and sequences are provided for *patched (ptc)* genes, including mammalian, e.g. human and mouse, and invertebrate homologs. Decreased expression of *ptc* is associated with the occurrence of human cancers, particularly basal cell carcinomas and other tumors of epithelial tissues such as the skin. The cancers may be familial, having as a component of risk a germline mutation in the gene, or may be sporadic. *Ptc*, and its antagonist *hedgehog*, are useful in creating transgenic animal models for these human cancers. The *ptc* nucleic acid compositions find use in identifying homologous or related genes; in producing compositions that modulate the expression or function of its encoded protein, *ptc*; for gene therapy; mapping functional regions of the protein- and in studying associated physiological pathways. In addition, modulation of the gene activity *in vivo* is used

for prophylactic and therapeutic purposes, such as treatment of cancer, identification of cell type based on expression, and the like. *Ptc*, anti-*ptc* antibodies and *ptc* nucleic acid sequences are useful as diagnostics for a genetic predisposition to cancer or developmental abnormality syndromes, and to identify specific cancers having mutations in this gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph having a restriction map of about 10 kbp of the 5' region upstream from the initiation codon of *Drosophila patched* gene and bar graphs of constructs of truncated portions of the 5' region joined to fl-galactosidase, where the constructs are introduced into fly cell lines for the production of embryos. The expression of fl-gal in the embryos is indicated in the right-hand table during early and late development of the embryo. The greater the number of +'s, the more intense the staining.

Fig. 2 shows a summary of mutations found in the human *patched* gene locus that are associated with basal cell nevus syndrome. Mutation (1) is found in sporadic basal cell carcinoma, and is a C to T transition in exon 3 at nucleotide 523 of the coding sequence, changing Leu 175 to Phe in the first extracellular loop. Mutations 2-4 are found in hereditary basal carcinoma nevus syndrome. (2) is an insertion of 9 bp at nucleotide 2445, resulting in the insertion of an additional 3 amino acids after amino acid 815. (3) is a deletion of 11 bp, which removes nt 2442-2452 from the coding sequence. The resulting frameshift truncates the open reading frame after amino acid 813, 'ust after the seventh transmembrane domain. (4) is a G to C alteration that changes two conserved nucleotides of the 3' splice site adjacent to exon 10, creating a non-functional splice site that truncates the protein after amino acid 449, in the second transmembrane region.

DATABASE REFERENCES FOR NUCLEOTIDE AND AMINO ACID SEQUENCES

The sequence for the *D. melanogaster patched* gene has the Genbank accession number M28418. The sequence for the mouse *patched* gene has the Genbank accession number lt30589-V46155. The sequence for the human *patched* gene has the Genbank accession number U59464.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Mammalian and invertebrate *patched (ptc)* gene compositions and methods for their isolation are provided. Of particular interest are the human and mouse homologs. Certain human cancers, e.g. basal cell carcinoma, transitional cell carcinoma of the bladder, meningiomas, medulloblastomas, etc., show decreased *ptc* activity, resulting from oncogenic mutations at the *ptc* locus. Many such cancers are sporadic, where the tumor cells have a somatic mutation in *ptc*. The basal cell nevus syndrome (BCNS), an inherited disorder, is associated with germline mutations in *ptc*. Such germline mutations may also be associated with other human cancers, including carcinomas, adenocarcinomas, sarcomas and the like. Decreased *ptc* activity is also associated with inherited developmental abnormalities, e.g. rib and craniofacial abnormalities, polydactyly, syndactyly and spina bifida.

The *ptc* genes and fragments thereof, encoded protein, and anti-*ptc* antibodies are useful in the identification of individuals predisposed to development of such cancers and developmental abnormalities, and in characterizing the phenotype of sporadic tumors that are associated with this gene, e.g., for diagnostic and/or prognostic benefit. The characterization is useful for prenatal screening, and in determining further treatment of the patient. Tumors may be typed or staged as to the *ptc* status, e.g. by detection of mutated sequences, antibody detection of abnormal protein products, and functional assays for altered *ptc* activity. The

encoded *ptc* protein is useful in drug screening for compositions that mimic *ptc* activity or expression, including altered forms of *ptc* protein, particularly with respect to *ptc* function as a tumor suppressor in oncogenesis.

The human and mouse *ptc* gene sequences and isolated nucleic acid compositions are provided. In identifying the mouse and human *patched* genes, cross-hybridization of DNA and amplification primers were employed to move through the evolutionary tree from the known *Drosophila ptc* sequence, identifying a number of invertebrate homologs. The human *patched* gene has been mapped to human chromosome band 9q22.3, and lies between the polymorphic markers D9Sl96 and D9S287 (a detailed map of human genome markers may be found in Dib *et al.* (1 996) Nature 280-152-1 http://www.genethon.fr).

DNA from a patient having a tumor or developmental abnormality, which may be associated with *ptc*, is analyzed for the presence of a predisposing mutation in the *ptc* gene. The presence of a mutated *ptc* sequence that affects the activity or expression of the gene product, *ptc*, confers an increased susceptibility to one or more of these conditions. Individuals are screened by analyzing their DNA for the presence of a predisposing oncogenic or developmental mutation, as compared to a normal sequence. A "normal" sequence of *patched* is provided in SEQ ID NO-.18 (human). Specific mutations of interest include any mutation that leads to oncogenesis or developmental abnormalities, including insertions, substitutions and deletions in the coding region sequence, introns that affect splicing, promoter or enhancer that affect the activity and expression of the protein.

Screening for tumors or developmental abnormalities may also be based on the functional or antigenic characteristics of the protein. Immunoassays designed to detect the normal or abnormal *ptc* protein may be used in screening. Where many diverse mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening

tools. Such assays may be based on detecting changes in the transcriptional regulation mediated by *ptc*, or may directly detect *ptc* transporter activity, or may involve antibody localization of *patched* in cells.

Inheritance of BCNS is autosomal dominant, although many cases are the result of new mutations. Diagnosis of BCNS is performed by protein, DNA sequence or hybridization analysis of any convenient sample from a patient, e.g. biopsy material, blood sample, scrapings from cheek, etc. A typical patient genotype will have a predisposing mutation on one chromosome. In tumors and at least sometimes developmentally affected tissues, loss of heterozygosity at the *ptc* locus leads to aberrant cell and tissue behavior. When the normal copy of *ptc* is lost, leaving only the reduced function mutant copy, abnormal cell growth and reduced cell layer adhesion is the result. Examples of specific *ptc* mutations in BCNS patients are a 9 bp insertion at nt 2445 of the coding sequence- and an 1 1 bp deletion of nt 2441 to 2452 of the coding sequence. These result in insertions or deletions in the region of the seventh transmembrane domain.

Prenatal diagnosis of BCNS may be performed, particularly where there is a family history of the disease, e.g. an affected parent or sibling. It is desirable, although not required, in such cases to determine the specific predisposing mutation present in affected family members. A sample of fetal DNA, such as an amniocentesis sample, fetal nucleated or white blood cells isolated from maternal blood, chorionic villus sample, etc. is analyzed for the presence of the predisposing mutation. Alternatively, a protein based assay, e.g. functional assay or immunoassay, is performed on fetal cells known to express *ptc*.

Sporadic tumors associated with loss of *ptc* function include a number of carcinomas and other transformed cells known to have deletions in the region of chromosome 9q22, e.g. basal cell carcinomas, transitional bladder cell carcinoma, meningiomas, medullomas, fibromas of the

heart and ovary, and carcinomas of the lung, ovary, kidney and esophagus. Characterization of sporadic tumors will generally require analysis of tumor cell DNA, conveniently with a biopsy sample. A wide range of mutations are found in sporadic cases, up to and including deletion of the entire long arm of chromosome 9. Oncogenic mutations may delete one or more exons, e.g. 8 and 9, may affect the amino acid sequence such as of the extracellular loops or transmembrane domains, may cause truncation of the protein by introducing a frameshift or stop codon, etc. Specific examples of oncogenic mutations include a C to T transition at nt 523-1 and deletions encompassing exon 9. C to T transitions are characteristic of ultraviolet mutagenesis, as expected with cases of skin cancer.

Biochemical studies may be performed to determine whether a candidate sequence variation in the *ptc* coding region or control regions is oncogenic. For example, a change in the promoter or enhancer sequence that downregulates expression of *patched* may result in predisposition to cancer. Expression levels of a candidate variant allele are compared to expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein; insertion of the variant control element into a vector with a reporter gene such as R-galactosidase, chloramphenical acetyltransferase, etc. that provides for convenient quantitation- and the like. The activity of the encoded *ptc* protein may be determined by comparison with the wild-type protein, e.g. by detection of transcriptional down-regulation of TGFP, *Wnt* family genes, *ptc* itself, or reporter gene fusions involving these target genes.

The human *patched* gene (SEQ ID NO:18) has a 4.5 kb open reading frame encoding a protein of 1447 amino acids. Including coding and noncoding sequences, it is about 89% identical at the nucleotide level to the mouse *patched* gene (SEQ ID NO-.09). The mouse *patched* gene (SEQ iD NO:09) encodes a protein (SEO ID NO:10) that has about 38% identical

amino acids to *Drosophila ptc* (SEQ ID NO:6), over about 1,200 amino acids. The butterfly homolog (SEQ ID NO:4) is 1,300 amino acids long and overall has a 50% amino acid identity to fly *ptc* (SEQ ID NO:6). A 267 bp exon from the beetle patched gene encodes an 89 amino acid protein fragment, which was found to be 44% and 51% identical to the corresponding regions of fly and butterfly *ptc* respectively.

The DNA sequence encoding *ptc* may be cDNA or genomic DNA or a fragment thereof. The term "*patched* gene" shall be intended to mean the open reading frame encoding specific *ptc* polypeptides, as well as adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 1 kb beyond the coding region, in either direction. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host.

The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons, 3' and 5' non-coding regions. Normally MRNA species have contiguous exons, with the intervening introns deleted, to create a continuous open reading frame encoding *ptc*.

The genomic *ptc* sequence has non-contiguous open reading frames, where introns interrupt the coding regions. A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It may further include the 3' and 5' untranslated regions found in the mature MRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb of flanking genomic DNA at either the 5' or 3' end of the coding region. The genomic DNA may be isolated as a fragment of 50 kbp or smaller; and substantially free

of flanking chromosomal sequence.

The nucleic acid compositions of the subject invention encode all or a part of the subject polypeptides. Fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by restriction enzyme digestion, by PCR amplification, etc. For the most part, DNA fragments will be of at least 15 nt, usually at least 18 nt, more usually at least about 50 nt. Such small DNA fragments are useful as primers for PCR, hybridization screening, etc. Larger DNA fragments, i.e. greater than 100 nt are useful for production of the encoded polypeptide. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject sequence under stringent conditions, as known in the art. It is preferable to chose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other.

The *ptc* genes are isolated and obtained in substantial purity, generally as other than an intact mammalian chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include a *ptc* sequence or fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", i.e. flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The DNA sequences are used in a variety of ways. They may be used as probes for identifying other patched genes. Mammalian homologs have substantial sequence similarity to the subject sequences, i.e. at least 75%, usually at least 90%, more usually at least 95%

sequence identity with the nucleotide sequence of the subject DNA sequence. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc. *A* reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul *et al.* (1990) J Mol Biol 215; 403-10.

Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0-9 M saline/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of homologous genes may be any mammalian species, e.g. primate species, particularly human-murines, such as rats and mice, canines, felines, bovines, ovines, equines, etc.

The DNA may also be used to identify expression of the gene in a biological specimen. The manner in which one probes cells for the presence of particular nucleotide sequences, as genomic DNA or RNA, is well-established in the literature and does not require elaboration here. Conveniently, a biological specimen is used as a source of MRNA. The MRNA may be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA strand, followed by polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, the MRNA sample is separated by gel electrophoresis, transferred to a suitable support, e.g.. nitrocellulose and then probed with a fragment of the subject DNA as a probe. Other techniques may also find use. Detection of MRNA having the subject sequence is indicative of *patched* gene expression in the sample.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid

cleaving agent, e.g. a chelated metal ion, such as iron or chromium for cleavage of the gene; as an antisense sequence-, or the like. Modifications may include replacing oxygen of the phosphate esters with sulfur or nitrogen, replacing the phosphate with phosphoramide, etc.

A number of methods are available for analyzing genomic DNA sequences. Where large amounts of DNA are available, the genomic DNA is used directly. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis, or amplified by conventional techniques, such as the polymerase chain reaction (PCR). The use of the polymerase chain reaction is described in Saiki, *et al.* (1 985) Science 239@487, and a review of current techniques may be found in Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, CSH Press 1989, pp.14.2-14.33.

A detectable label may be included in the amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-Xrhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ^{32}P, ^{35}S, ^{3}H; etc. The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label Into the amplification product.

The amplified or cloned fragment may be sequenced by dideoxy or other methods, and the sequence of bases compared to the normal *ptc* sequence. Hybridization with the variant sequence may also be used to determine its presence, by Southern blots, dot blots, etc. Single

strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence variation as alterations in electrophoretic mobility. The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilized on a solid support, as described in WO 95/11995, may also be used as a means of detecting the presence of variant sequences. Alternatively, where a predisposing mutation creates or destroys a recognition site for a restriction endonuclease, the fragment is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel electrophoresis, particularly acrylamide or agarose gels.

The subject nucleic acids can be used to generate transgenic animals or site specific gene modifications in cell lines. Transgenic animals may be made through homologous recombination, where the normal patched locus is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome, Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACS, and the like.

The modified cells or animals are useful in the study of *patched* function and regulation. For example, a series of small deletions and/or substitutions may be made in the *patched* gene to determine the role of different exons in oncogenesis, signal transduction, etc. Of particular interest are transgenic animal models for carcinomas of the skin, where expression of *ptc* is specifically reduced or absent in skin cells. An alternative approach to transgenic models for this disease are those where one of the mammalian *hedgehog* genes, *e.g. Shh, Ihh, Dhh,* are upregulated in skin cells, or in other cell types. For models of skin abnormalities, one may use a skin-specific promoter to drive expression of the transgene, or other inducible promoter that can be regulated in the animal model. Such promoters include keratin gene promoters. Sp cific constructs of interest include anti-sense *ptc*, which will block *ptc* expression, expression of

dominant negative *ptc* mutations, and over-expression of HH genes. A detectable marker, such as *lacZ* may be introduced into the *patched* locus, where upregulation of patched expression will result in an easily detected change in phenotype.

One may also provide for expression of the *patched* gene or variants thereof in cells or tissues where it is not normally expressed or at abnormal times of development. Thus, mouse models of spina bifida or abnormal motor neuron differentiation in the developing spinal cord are made available. In addition, by providing expression of *ptc* protein in cells in which it is otherwise not normally produced, one can induce changes in cell behavior, e.g. through *ptc* mediated transcription modulation.

DNA constructs for homologous recombination will comprise at least a portion of the *patched or hedgehog* gene with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown *et al.* (1 990) Methods in Enzymoloay 185:527-537.

For embryonic stem (ES) cells, an ES cell line may be employed, or ES cells may be obtained freshly from a host, e.g. mouse, rat, guinea pig, etc. Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used

for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected.

The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogeneic or congenic grafts or transplants, or in *in vitro* culture. The transgenic animals may be any non-human mammal, such as laboratory animals, domestic animals, etc. The transgenic animals may be used in functional studies, drug screening, etc., e.g. to determine the effect of a candidate drug on basal cell carcinomas.

The subject gene may be employed for producing all or portions of the patched protein. For expression, an expression cassette may be employed, providing for a transcriptional and translational initiation region, which may be inducible or constitutive, the coding region under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. Various transcriptional initiation regions may be employed which are functional in the expression host.

Specific *ptc* peptides of interest include the extracellular domains, particularly in the human mature protein, aa 120 to 437, and aa 770 to 1027. These peptides may be used as immunogens to raise antibodies that recognize the protein in an intact cell membrane. The cytoplasmic domains, as shown in Figure 2, (the amino terminus and carboxy terminus) are of interest in binding assays to detect ligands involved in signaling mediated by *ptc*.

The peptide may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism or cells of a higher organism, e.g. eukaryotes such as vertebrates, particularly mammals, may be used as the expression host, such as *E. coli, B, subthis, S. cerevisiae,* and the like. In many situations, it may be desirable to express the *patched* gene in a mammalian host, whereby the *patched* gene will be glycosylated, and transported to the cellular membrane for various studies.

With the availability of the protein in large amounts by employing an expression host, the protein may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified protein will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to and including 100% pure. By pure is intended free of other proteins, as well as cellular debris.

The polypeptide is used for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, whereas larger fragments or the entire gene allow for the production of antibodies over the surface of the polypeptide or protein. Antibodies may be raised to the normal or mutated forms of *ptc*- The extracellular domains of the protein are of interest as epitopes, particular antibodies that recognize common changes found in abnormal, oncogenic *ptc*, which compromise the protein activity. Antibodies may be raised to isolated peptides corresponding to these domains, or to the native protein, e.g. by immunization with cells expressing *ptc*, immunization with liposomes having *ptc* inserted in the membrane, etc. Antibodies that recognize the extracellular domains of *ptc* are useful in diagnosis, typing and staging of human carcinomas.

Antibodies are prepared in accordance with conventional ways, where the expressed polypeptide or protein may be used as an immunogen, by itself or conjugated to known immunogenic carriers, e.g. KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate, For monoclonal antibodies, after one or more booster injections, the spleen may be isolated, the splenocytes immortalized, and then screened for high affinity antibody binding. The immortalized cells, e.g. hybridomas, producing the desired antibodies may then be expanded. For further description, see Monoclonal Antibodies- A Laboratory Manual, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1988. If desired, the MRNA encoding the heavy and light chains may be isolated and mutagenized by cloning in *E. coli*, and the heavy and light chains may be mixed to further enhance the affinity of the antibody.

The antibodies find particular use in diagnostic assays for developmental abnormalities, basal cell carcinomas and other tumors associated with mutations in *ptc*. Staging, detection and typing of tumors may utilize a quantitative immunoassay for the presence or absence of normal *ptc*. Alternatively, the presence of mutated forms of *ptc* may be determined. A reduction in normal *ptc* and/or presence of abnormal *ptc* is indicative that the tumor is *ptc*-associated.

A sample is taken from a patient suspected of having a *ptc*-associated tumor, developmental abnormality or BCNS. Samples, as used herein, include biological fluids such as blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid and the like- organ or tissue culture derived fluids, and fluids extracted from physiological tissues. Also included in the term are derivatives and fractions of such fluids. Biopsy samples are of particular interest, e.g. skin lesions, organ tissue fragments, etc. Where metastasis is suspected, blood samples may be preferred. The number of cells in a sample will generally be at least about 103, usually at least 104 more usually at least about 105. The cells may be dissociated, in the case of solid tissues,

or tissue sections may be analyzed. Alternatively a lysate of the cells may be prepared.

Diagnosis may be performed by a number of methods. The different methods all determine the absence or presence of normal or abnormal *ptc* in patient cells suspected of having a mutation in *ptc*. For example, detection may utilize staining of intact cells or histological sections, performed in accordance with conventional methods. The antibodies of interest are added to the cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemiluminescers, or other labels for direct detection. Alternatively, a second stage antibody or reagent is used to amplify the signal. Such reagents are well-known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, etc.

An alternative method for diagnosis depends on the *in vitro* detection of binding between antibodies and *ptc* in a lysate. Measuring the concentration of *ptc* binding in a sample or fraction thereof may be accomplished by a variety of specific assays. A conventional sandwich type assay may be used. For example, a sandwich assay may first attach *ptc*-specific antibodies to an insoluble surface or support. The particular manner of binding is not crucial so long as it is compatible with the reagents and overall methods of the invention They may be bound to the plates covalently or non-covalently, preferably non-covalently.

The insoluble supports may be any compositions to which polypeptides can be bound, which is readily separated from soluble material, and which is otherwise compatible with the overall method. The surface of such supports may be solid or porous and of any convenient

shape. Examples of suitable insoluble supports to which the receptor is bound include beads, e.g. magnetic beads, membranes and microtiter plates. These are typically made of glass, plastic (e.g. polystyrene), polysaccharides, nylon or nitrocellulose. Microtiter plates are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples.

Patient sample lysates are then added to separately assayable supports (for example, separate wells of a microtiter plate) containing antibodies. Preferably, a series of standards, containing known concentrations of normal and/or abnormal *ptc* is assayed in parallel with the samples or aliquots thereof to serve as controls. Preferably, each sample and standard will be added to multiple wells so that mean values can be obtained for each. The incubation time should be sufficient for binding, generally, from about 0.1 to 3 hr is sufficient. After incubation, the insoluble support is generally washed of non-bound components. Generally, a dilute non-ionic detergent medium at an appropriate pH, generally 7-8, is used as a wash medium. From one to six washes may be employed, with sufficient volume to thoroughly wash nonspecifically bound proteins present in the sample.

After washing, a solution containing a second antibody is applied. The antibody will bind *ptc* with sufficient specificity such that it can be distinguished from other components present. The second antibodies may be labeled to facilitate direct, or indirect quantification of binding. Examples of labels that permit direct measurement of second receptor binding include radiolabels, such aS 3H or 1251, fluorescers, dyes, beads, chemilumninescers, colloidal particles, and the like. Examples of labels which permit indirect measurement of binding include enzymes where the substrate may provide for a colored or fluorescent product. In a preferred embodiment, the antibodies are labeled with a covalently bound enzyme capable of providing a detectable product signal after addition of suitable substrate. Examples of suitable enzymes

for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such antibody-enzyme conjugates are readily produced by techniques known to those skilled in the art. The incubation time should be sufficient for the labeled ligand to bind available molecules. Generally, from about 0. 1 to 3 hr is sufficient, usually 1 hr sufficing.

After the second binding step, the insoluble support is again washed free of non-specifically bound material. The signal produced by the bound conjugate is detected by conventional means. Where an enzyme conjugate is used, an appropriate enzyme substrate is provided so a detectable product is formed.

Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for *ptc* as desired, conveniently using a labeling method as described for the sandwich assay.

Other diagnostic assays of interest are based on the functional properties of *ptc* protein itself. Such assays are particularly useful where a large number of different sequence changes lead to a common phenotype, i.e., loss of protein function leading to oncogenesis or developmental abnormality. For example, a functional assay may be based on the transcriptional changes mediated by *hedgehog and patched* gene products. Addition of soluble Hh to embryonic stem cells causes induction of transcription in target genes. The presence of functional *ptc* can be determined by its ability to antagonize Hh activity. Other functional assays may detect the transport of specific molecules mediated by *ptc*, in an intact cell or membrane fragment. Conveniently, a labeled substrate is used, where the transport in or out of the cell can be quantitated by radiography, microscopy, flow cytometry, spectrophotometry, etc. Other assays may detect conformational changes, or changes in the subcellular localization of *patched*

protein.

By providing for the production of large amounts of patched protein, one can identify ligands or substrates that bind to, modulate or mimic the action of *patched*. A common feature in basal cell carcinoma is the loss of adhesion between epidermal and dermal layers, indicating a role for *ptc* in maintaining appropriate cell adhesion. Areas of investigation include the development of cancer treatments, wound healing, adverse effects of aging, metastasis, etc.

Drug screening identifies agents that provide a replacement for *ptc* function in abnormal cells. The role of *ptc* as a tumor suppressor indicates that agents which mimic its function, in terms of transmembrane transport of molecules, transcriptional down-regulation, etc., will inhibit the process of oncogenesis. These agents may also promote appropriate cell adhesion in wound healing and aging, to reverse the loss of adhesion observed in metastasis, etc. Conversely, agents that reverse *ptc* function may stimulate controlled growth and healing. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions, transporter function, etc.

The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of *patched*. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than

50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty 'ds, steroids, purines, pyrimidines, derivatives, structural analogs or a combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, enzymes, specific binding molecules, particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce nonspecific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4° and 40° C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Other assays of interest detect agents that mimic patched function, such as repression of target gene transcription, transport of patched substrate compounds, etc. For example, an expression construct comprising a *patched* gene may be introduced into a cell line under conditions that allow expression. The level of patched activity is determined by a functional assay, as previously described. In one screening assay, candidate agents are added in combination with a Hh protein, and the ability to overcome Hh antagonism of *ptc* is detected. In another assay, the ability of candidate agents to enhance *ptc* function is determined. Alternatively, candidate agents are added to a cell that lacks functional *ptc*, and screened for the ability to reproduce *ptc* in a functional assay.

The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host for treatment of cancer or developmental abnormalities attributable to a defect in *patched* function. The compounds may also be used to enhance patched function in wound healing, aging, etc. The inhibitory agents may be administered in a variety of ways, orally, topically, parenterally e.g. subcutaneously, intraperitoneally, by viral infection, intravascularly, etc. Topical treatments ar of particular

interest. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt.%.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

The gene or fragments thereof may be used as probes for identifying the 5' non-coding region comprising the transcriptional initiation region, particularly the enhancer regulating the transcription of *patched*. By probing a genomic library, particularly with a probe comprising the 5' coding region, one can obtain fragments comprising the 5' non-coding region. If necessary, one may walk the fragment to obtain further 5' sequence to ensure that one has at least a functional portion of the enhancer. It is found that the enhancer is proximal to the 5' coding region, a portion being in the transcribed sequence and downstream from the promoter sequences. The transcriptional initiation region may be used for many purposes, studying embryonic development, providing for regulated expression of *patched* protein or other protein of interest during embryonic development or thereafter, and in gene therapy.

The gene may also be used for gene therapy. Vectors useful for introduction of the gene include plasmids and viral vectors. Of particular interest are retroviral-based vectors, e.g. moloney murine leukemia virus and modified human immunodeficiency virus- adenovirus vectors, etc. Gene therapy may be used to treat skin lesions, an affected fetus, etc., by

transfection of the normal gene into embryonic stem cells or into other fetal cells. A wide variety of viral vectors can be employed for transfection and stable integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.* (1991) Science 254:1509-1512 and Smith *et al.* (1 990) Molecular and Cellular Biology 3268-3271.

The following examples are offered by illustration not by way of limitation.

EXPERIMENTAL

Methods and Materials

PCR on Mosquito (Anopheles gambiae) Genomic DNA. PCR primers were based on amino acid stretches of fly *ptc* that were not likely to diverge over evolutionary time and were of low degeneracy. Two such primers (P2RI (SEO ID NO-14)-GGACGAATTCAARGTNCAYCARYTNTGG, P4RI: (SEQ ID NO:15) GGACGAATTCCYTCCCARAARCANTC, (the underlined sequences are Eco RI linkers) amplified an appropriately sized band from mosquito genomic DNA using the PCR. The program conditions were as follows:

94°C 4 min.; 72°C Add Taq;
[49°C 30 sec.; 72°C 90 sec.; 94°C 15 sec] 3 times
[94°C 15 sec.; 50°C 30 sec.; 72°C 90 sec] 35 times
72 °C 10 min; 4°C hold

This band was subcloned into the EcoRV site of pBluescript II and sequenced using the USB Sequence kit.

Screen of a Butterfly cDNA Library with Mosquito PCR Product. Using the mosquito PCR product (SEQ ID NO:7) as a probe, a 3 day embryonic *Precis coenia* λgt10 cDNA library (generously provided by Sean Carroll) was screened. Filters were hybridized at 65° C overnight in a solution containing 5xSSC, 10% dextran sulfate, 5x Denhardt's, 200 µg/ml sonicated

salmon sperm DNA, and 0.5% SDS. Filters were washed in 0.1X SSC, 0.1% SDS at room temperature several times to remove nonspecific hybridization. Of the 100,000 plaques initially screened, 2 overlapping clones, Ll and L2, were isolated, which corresponded to the N terminus of butterfly *ptc*. Using L2 as a probe, the library filters were rescreened and 3 additional clones (L5, L7, L8) were isolated which encompassed the remainder of the *ptc* coding sequence. The full length sequence of butterfly *ptc* (SEQ ID NO:3) was determined by ABI automated sequencing.

Screen of a Tribolium (beetle) Genomic Library with Mosquito PCR Product and 900 bp Fragment from the Butterfly Clone. A λgem11 genomic library from *Tribolium casteneum* (gift of Rob Dennell) was probed with a mixture of the mosquito PCR (SEQ ID NO:7) product and BstXI/EcoRI fragment of L2. Filters were hybridized at 55° C overnight and washed as above. Of the 75,000 plaques screened, 14 clones were identified and the SacI fragment of T8 (SEQ ID NO:1), which crosshybridized with the mosquito and butterfly probes, was subcloned into pBluescript.

PCR on Mouse cDNA Using Degenerate Primers Derived from Regions Conserved in the Four Insect Homologues. Two degenerate PCR primers (P4REV- (SEQ ID NO:16) GGACGAATTCYTNGANTGYTTYTGGGA- P22- (SEQ ID NO:17) CATACCAGCCAAG CTTGTCIGGCCARTGCAT) were designed based on a comparison of *ptc* amino acid sequences from fly *(Drosophila melanogaster)* (SEQ ID NO:6), mosquito *(Anopheles gambiae)* (SEQ ID NO:8), butterfly (*Precis coenia*) (SEQ ID NO:4), and beetle *(Tribolium casteneum)* (SEQ ID NO:2). I represents inosine, which can form base pairs with all four nucleotides. P22 was used to reverse transcribe RNA from 12.5 dpc mouse limb bud (gift from David Kingsley) for 90 min at 37° C. PCR using P4REV (SEQ ID NO:17) and P22 (SEQ ID NO:18) was then performed on 1 μl of the resultant cDNA under the following conditions:

94°C 4 min.; 72°C Add Taq;
[94 °C 15 sec.- 50 °C 30 sec.- 72 °C 90 sec.] 35 times
72 °C 10 min.-, 4 °C hold

PCR products of the expected size were subcloned into the TA vector (Invitrogen) and sequenced with the Sequenase Version 2.0 DNA Sequencing Kit (U. S. B.).

Using the cloned mouse PCR fragment as a probe, 300,000 plaques of a mouse 8.5 dpc λgtl0 cDNA library (a gift from Brigid Hogan) were screened at 65° C as above and washed in 2x SSC, 0.1% SDS at room temperature. 7 clones were isolated, and three (M2, M4, and M8) were subcloned into pBluescript II. 200,000 plaques of this library were rescreened using first, a 1.1 kb EcoRI fragment from M2 to identify 6 clones (M9-MI6) and secondly a mixed probe containing the most N terminal (Xhol fragment from M2) and most C terminal sequences (BamHI/BgIII fragment from M9) to isolate 5 clones (MI7-M21). M9, M10, M14, and M17-21 were subcloned into the EcoRI site of pBluescript II (Strategene).

RNA Blots and in situ Hybridizations in Whole and Sectioned Mouse Embryos:

Northerns. A mouse embryonic Northern blot and an adult multiple tissue Northern blot (obtained from Clontech) were probed with a 900 bp EcoRI fragment from an N terminal coding region of mouse *ptc*. Hybridization was performed at 65° C in 5x SSPE, l0x Denhardt's, 100 µg/ml sonicated salmon sperm DNA, and 2% SDS. After several short room temperature washes in 2x SSC, 0.05% SDS, the blots were washed at high stringency in 0. 1 X SSC, 0.1% SDS at 50° C.

In situ hybridization of sections: 7.75, 8.5, 11.5, and 13.5 dpc mouse embryos were dissected in PBS and frozen in Tissue-Tek medium at -80° C. 12-16 µm frozen sections were cut, collected onto VectaBond (Vector Laboratories) coated slides, and dried for 30-60 minutes at room temperature. After a 10 minute fixation in 4% paraformaldehyde in PBS, the slides

were washed 3 times for 3 minutes in PBS, acetylated for 10 minutes in 0.25% acetic anhydride in triethanolamine, and washed three more times for 5 minutes in PBS. Prehybridization (50% formamide, 5X SSC, 250 µg/ml yeast tRNA, 500 µg/ml sonicated salmon sperm DNA, and 5x Denhardt's) was carried out for 6 hours at room temperature in 50% formamide/5x SSC humidified chambers. The probe, which consisted of 1 kb from the N-terminus of *ptc*, was added at a concentration of 200-1000 ng/ml into the same solution used for prehybridization, and then denatured for five minutes at 80° C. Approximately 75 µl of probe were added to each slide and covered with Parafilm. The slides were incubated overnight at 65° C in the same humidified chamber used previously. The following day, the probe was washed successively in 5X SSC (5 minutes, 65° C), 0.2X SSC (1 hour, 65° C), and 0.2X SSC (10 minutes, room temperature). After five minutes in buffer Bl (0.1M maleic acid, 0.15 M NaCl, pH 7.5), the slides were blocked for 1 hour at room temperature in 1% blocking reagent (Boerhinger-Mannheim) in buffer Bl, and then incubated for 4 hours in buffer Bl containing the DIG-AP conjugated antibody (Boerhinger-Mannheim) at a 1:5000 dilution. Excess antibody was removed during two 15 minute washes in buffer Bl, followed by five minutes in buffer B3 (100 mM Tris, 100mM NaCl, 5mM $MgCl_2$, pH 9.5). The antibody was detected by adding an alkaline phosphatase substrate (350 µl 75 mg/ml X-phosphate in DMF, 450 µl 50 mg/ml NBT in 70% DMF in 100 mls of buffer B3) and allowing the reaction to proceed overnight in the dark. After a brief rinse in 10 mM Tris, 1mM EDTA, pH 8.0, the slides were mounted with Aquamount (Lerner Laboratories).

Drosophila 5-transcriptional initiation region β-gal constructs. A series of constructs were designed that link different regions of the *ptc* promoter from *Drosophila* to a LacZ reporter gene in order to study the cis regulation of the *ptc* expression pattern. See Fig. 1. A 10.8kb BamHI/BspMI fragment comprising the 5'-non-coding region of the MRNA at its 3'-

terminus was obtained and truncated by restriction enzyme digestion as shown in Fig. 1. These expression cassettes were introduced into *Drosophila* lines using a P-element vector (Thummel *et al.* (1 988) Gene- 74:445-456), which were injected into embryos, providing flies which could be grown to produce embryos. (See Spradling and Rubin (1982) Science 218:341-347 for a description of the procedure.) The vector used a pUC8 background into which was introduced the white gene to provide for yellow eyes, portions of the P-element for integration, and the constructs were inserted into a polylinker upstream from the LacZ gene. The resulting embryos, larvae, and adults were stained using antibodies to LacZ protein conjugated to HRP and the samples developed with OPD dye to identify the expression of the LacZ gene. The staining pattern in embryos is described in Fig. 1, indicating whether there was staining during the early and late development of the embryo.

Isolation of a Mouse ptc Gene. Homologues of fly *ptc* (SEQ ID NO:6) were isolated from three insects: mosquito, butterfly and beetle, using either PCR or low stringency library screens. PCR primers to six amino acid stretches of *ptc* of low mutatability and degeneracy were designed. One primer pair, P2 and P4, amplified an homologous fragment of *ptc* from mosquito genomic DNA that corresponded to the first hydrophilic loop of the protein. The 345bp PCR product (SEQ ID NO:7) was subcloned and sequenced and when aligned to fly *ptc*, showed 67% amino acid identity.

The cloned mosquito fragment was used to screen a butterfly λgt 10 cDNA library. Of 100,000 plaques screened, five overlapping clones were isolated and used to obtain the full length coding sequence. The butterfly *ptc* homologue (SEQ ID NO:4) is 1,311 amino acids long and overall has 50% amino acid identity (72% similarity) to fly *ptc*. With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. The mosquito PCR clone (SEQ ID NO:7) and a corresponding fragment of butterfly cDNA were

used to screen a beetle λgemII genomic library. Of the plaques screened, 14 clones were identified. A fragment of one clone (T8), which hybridized with the original probes, was subcloned and sequenced. This 3kb piece contains an 89 amino acid exon (SEQ ID NO:2) which is 44% and 51% identical to the corresponding regions of fly and butterfly *ptc* respectively.

Using an alignment of the four insect homologues in the first hydrophilic loop of the *ptc*, two PCR primers were designed to a five and six amino acid stretch which were identical and of low degeneracy. These primers were used to isolate the mouse homologue using RT-PCR on embryonic limb bud RNA. An appropriately sized band was amplified and upon cloning and sequencing, it was found to encode a protein 65% identical to fly *ptc*. Using the cloned PCR product and subsequently, fragments of mouse *ptc* cDNA, a mouse embryonic λcDNA library was screened. From about 300,000 plaques, 17 clones were identified and of these, 7 form overlapping cDNA's that comprise most of the protein-coding sequence (SEQ ID NO:9) .

Developmental and Tissue Distribution of Mouse ptc RNA. In both the embryonic and adult Northern blots, the *ptc* probe detects a single 8kb message. Further exposure does not reveal any additional minor bands. Developmentally, *ptc* mRNA is present in low levels as early as 7 dpc and becomes quite abundant by 11 and 15 dpc. While the gene is still present at 17 dpc, the Northern blot indicates a clear decrease in the amount of message at this stage. In the adult, *ptc* RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle, and testes.

In situ Hybridization of Mouse ptc in Whole and Section Embryos. Northern analysis indicates that *ptc* mRNA is present at 7 dpc, while there is no detectable signal in sections from 7.75 dpc embryos. This discrepancy is explained by the low level of transcription. In contrast, *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be

detected in the developing lung buds and gut, consistent with its adult Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system, as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective sclerotome and eventually forms bone in the vertebral column. *ptc* is present in a wide range of tissues from endodermal, mesodermal and ectodermal origin supporting its fundamental role in embryonic development.

Isolation of the Human ptc Gene. To isolate human *ptc* (h*ptc*), 2 x 10^5 plaques from a human lung cDNA library (HL3022a, Clonetech) were screened with a lkbp mouse *ptc* fragment, M2-2. Filters were hybridized overnight at reduced stringency (60° C in 5X SSC, 10% dextran sulfate, 5X Denhardt's, 0.2 mg/ml sonicated salmon sperm DNA, and 0.5% SDS). Two positive plaques (Hl and H2) were isolated, the inserts cloned into pBluescript, and upon sequencing, both contained sequence highly similar to the mouse *ptc* homolog. To isolate the 5' end, an additional 6 x 10^5 plaques were screened in duplicate with M2-3 EcoRI and M2-3 Xho I (containing 5' untranslated sequence of mouse *ptc*) probes. Ten plaques were purified and of these, inserts were subcloned into pBluescript. To obtain the full coding sequence, H2 was fully and H14, H20, and H21 were partially sequenced. The 5.lkbp of human *ptc* sequence (SEQ ID NO:18) contains an open reading frame of 1447 amino acids (SEQ ID NO:19) that is 96% identical and 98% similar to mouse *ptc*. The 5' and 3' untranslated sequences of human *ptc* (SEQ ID NO:18) are also highly similar to mouse *ptc* (SEQ ID NO:19) suggesting conserved regulatory sequence.

Comparison of Mouse, Human, Fly and Butterfly Sequences. The deduced mouse *ptc* protein sequence (SEQ ID NO:10) has about 38% identical amino acids to fly *ptc* over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein

excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly protein. Based on the sequence conservation of *ptc* and the functional conservation of *hedgehog* between fly and mouse, one concludes that *ptc* functions similarly in the two organisms. A comparison of the amino acid sequences of mouse (m*ptc*) (SEQ ID NO:10), human (h*ptc*) (SEQ ID NO:19), butterfly (b*ptc*)(SEQ ID NO:4) and *drosophila* (*ptc*) (SEQ ID NO:6) is shown in Table 1.

TABLE 1

ALIGNMENT OF HUMAN, MOUSE, FLY, AND BUTTERFLY *PTC* HOMOLOGS

```
HPTC    MASAGNAAEPQDR--GGGGSGCIGAPGRPAGGGRRRRTGGLRRAAAPDRDYLHRPSYCDA
MPTC    MASAGNAA---------------GALGRQAGGGRRRRTGGPHRA-APDRDYLHRPSYCDA
PTC     M-----DRDSLPRVPDTHGD--VVDE---------KLFSDL---------YI-RTSWVDA
BPTC    MVAPDSEAPSNPRITAAHESPCATEA---------RHSADL---------YI-RTSWVDA
        *                                    .  ..          *. * * **

HPTC    AFALEQISKGKATGRKAPLWLRAKFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFAVGLKA
MPTC    AFALEQISKGKATGRKAPLWLRAKFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFAVGLKA
PTC     QVALDQIDKGKARGSRTAIYLRSVFQSHLETLGSSVQKHAGKVLFVAILVLSTFCVGLKS
BPTC    ALALSELEKGNIEGGRTSLWIRAWLQEQLFILGCFLQGDAGKVLFVAILVLSTFCVGLKS
          ** .. **.  * .... .*. .*  *  **   * . ** * *..*....* ****.

HPTC    ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
MPTC    ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
PTC     AQIHSKVHQLWIQEGGRLEAELAYTQKTIGEDESATHQLLIQTTHDPNASVLHPQALLAH
BPTC    AQIHTRVDQLWVQEGGRLEAELKYTAQALGEADSSTHQLVIQTAKDPDVSLLHPGALLEH
        *.... *..**.. ***.  ** ** . .**      **.***  .   ..*   *** *

HPTC    LDSALQASRVHVYMYNRQWKLEHLCYKSGELITET-GYMDQIIEYLYPCLIITPLDCFWE
MPTC    LDSALQASRVHVYMYNRQWKLEHLCYKSGELITET-GYMDQIIEYLYPCLIITPLDCFWE
PTC     LEVLVKATAVKVHLYDTEWGLRDMCNMPSTPSFEGIYYIEQILRHLIPCSIITPLDCFWE
BPTC    LKVVHAATRVTVHMYDIEWRLKDLCYSPSIPDFEGYHHIESIIDNVIPCAIITPLDCFWE
        *     *. * * .*. .* * ..*  ..    *    .. *. . ** **********

HPTC    GAKLQSGTAYLLGKPPLR----WTNFDPLEFLEELK------KINYQVDSWEEMLNKAEV
MPTC    GAKLQSGTAYLLGKPPLR----WTNFDPLEFLEELK------KINYQVDSWEEMLNKAEV
PTC     GSQLL-GPESAVVIPGLNQRLLWTTLNPASVMQYMKQKMSEEKISFDFETVEQYMKRAAI
BPTC    GSKLL-GPDYPIYVPHLKHKLQWTHLNPLEVVEEVK-KL---KFQFPLSTIEAYMKRAGI
         *..* *     *  * *     ** ..*   .. .*      *. .   . *  ...*.

HPTC    GHGYMDRPCLNPADPDCPATAPNKNSTKPLDMALVLNGGCHGLSRKYMHWQEELIVGGTV
MPTC    GHGYMDRPCLNPADPDCPATAPNKNSTKPLDVALVLNGGCQGLSRKYMHWQEELIVGGTV
PTC     GSGYMEKPCLNPLNPNCPDTAPNKNSTQPPDVGAILSGGCYGYAAKHMHWPEELIVGGRK
BPTC    TSAYMKKPCLDPTDPHCPATAPNKKSGHIPDVAAELSHGCYGFAAAYMHWPEQLIVGGAT
          .** .***.* .*.** *****.*     *..  *. ** * .   *** *.*****
HPTC    KNSTGKLVSAHALQTMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQRTYVEVV
MPTC    KNATGKLVSAHALQTMFQLMTPKQMYEHFRGYDYVSHINWNEDRAAAILEAWQRTYVEVV
```

```
PTC     RNRSGHLRKAQALQSVVQLMTEKEMYDQWQDNYKVHHLGWTQEKAAEVLNAWQRNFSREV
BPTC    RNSTSALRSARALQTVVQLMGEREMYEYWADHYKVHQIGWNQEKAAAVLDAWQRKFAAEV
        .* .. *  *.***.. ***  ..**.   .   * .. * ...** .*.**** .   *

HPTC    HQSVAQNSTQK----VLSFTTTTLDDILKSFSDVSVIRVASGYLLMLAYACLTMLRW-DC
MPTC    HQSVAPNSTQK----VLPFTTTTLDDILKSFSDVSVIRVASGYLLMLAYACLTMLRW-DC
PTC     EQLLRKQSRIATNYDIYVFSSAALDDILAKFSHPSALSIVIGVAVTVLYAFCTLLRWRDP
BPTC    RKI-TTSGSVSSAYSFYPFSTSTLNDILGKFSEVSLKNIILGYMFMLIYVAVTLIQWRDP
         .     .          *....*.***  **. *   .  *    . *   *...* *

HPTC    SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF
MPTC    SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF
PTC     VRGQSSVGVAGVLLMCFSTAAGLGLSALLGIVFNAASTQVVPFLALGLGVDHIFMLTAAY
BPTC    IRSQAGVGIAGVLLLSITVAAGLGFCALLGIPFNASSTQIVPFLALGLGVQDMFLLTHTY

HPTC    SETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFFMAALIPIPALRAFSLQAAVVVV
MPTC    SETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFFMAALIPIPALRAFSLQAAVVVV
PTC     AESN------RREQTKLILKKVGPSILFSACSTAGSFFAAAFIPVPALKVFCLQAAIVMC
BPTC    VEQAGD--VPREERTGLVLKKSGLSVLLASLCNVMAFLAAALLPIPAFRVFCLQAAILLL

HPTC    FNFAMVLLIFPAILSMDLYRREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPP
MPTC    FNFAMVLLIFPAILSMDLYRPEDRRLDIFCCFTSPCVSRVIQVEPQAYTEPHSNTRYSPP
PTC     SNLAAALLVFPAMISLDLRRRTAGRADIFCCCF-PVWKEQPKVAPPVLPLNNNNGR----
BPTC    PNLGSILLVFPAMISLDLRRRSAAPADLLCCLM-P---ESP------LPKKKIPER----

HPTC    PPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDT LSCQSP
MPTC    PPYTSHSFAHETHITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDNLSCQSP
PTC     ------ ---------------------------- GARHPKSCNNNRVPLPAQNPLLEQPA
BPTC    ---------------------------------- AKTRKNDKTHRID-TTRQPLDPDVS

HPTC    ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSSFAEKHYAPFLLKPKAKVVVIFLFLGLLG
MPTC    ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSSFAEKHYAPFLLKPKAKVVVILLFLGLLG
PTC     DIPGSS------------HSLASF----SLATFAFQHYTPFLMRSWVKFLTVMGFLAALI
BPTC    ENVTKT------------CCL-SV----SLTKWAKNQYAPFIMRPAVKVTSMLALIAVIL

HPTC    VSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNMYIVTQKA-DYPNIQHLLYD
PTC     SSLYASTRLQDGLDIIDLVPKDSNEHKFLDAQTRLFGFYSMYAVTQGNFEYPTQQQLLRD
BPTC    TSVWGATKVKDGLDLTDIVPENTDEHEFLSRQEKYFGFYNMYAVTQGNFEYPTNQKLLYE

HPTC    LHRSFSNVKYVMLEENKQLPKMWLHYFRDWLQGLQDAFDSDWETGKIMPNN-YKNGSDDG
MPTC    LHKSFSNVKYVMLEENKQLPQMWLHYFRDWLQGLQDAFDSDWETGRIMPNN-YKNGSDDG
PTC     YHDSFVRVPHVIKNDNGGLPDFWLLLFSEWLGNLQKIFDEEYRDGRLTKECWFPNASSDA
BPTC    YHDQFVRIPNIIKNDNGGLTKFWLSLFRDWLLDLQVAFDKEVASGCITQEYWCKNASDEG

HPTC    VLAYKLLVQTGSRDKPIDISQLTK-QRLVDADGIINPSAFYIYLTAWVSNDPVAYAASQA
MPTC    VLAYKLLVQTGSRDKPIDISQLTK-QRLVDADGIINPSAFYIYLTAWVSNDPVAYAASQA
PTC     ILAYKLIVQTGHVDNPVDKELVLT-NRLVNSDGIINQRAFYNYLSAWATNDVFAYGASQG
BPTC    ILAYKLMVQTGHVDNPIDKSLITAGHRLVDKDGIINPKAFYNYLSAWATNDALAYGASQG
```

```
HPTC    NIRPHRPEWVHDKADYMPETRLRIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICS
MPTC    NIRPHRPEWVHDKADYMPETRLRIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRVICN
PTC     KLYPEPRQYFHQPNEY----DLKIPKSLPLVYAQMPFYLHGLTDTSQIKTLIGHIRDLSV
BPTC    NLKPQPQRWIHSPEDV----HLEIKKSSPLIYTQLPFYLSGLSDTDSIKTLIRSVRDLCL

HPTC    NYTSLGLSSYPNGYPFLFWEQYIGLPHWLLLFISVVLACTFLVCAVFLLNPWTAGIIVMV
MPTC    NYTSLGLSSYPNGYPFLFWEQYISLRHWLLLSISVVLACTFLVCAVFLLNPWTAGIIVMV
PTC     KYEGFGLPNYPSGIPFIFWEQYMTLRSSLAMILACVLLAALVLVSLLLLSVWAAVLVILS
BPTC    KYEAKGLPNFPSGIPFLFWEQYLYLRTSLLLALACALGAVFIAVMVLLLNAWAAVLVTLA

HPTC    LALMTVELFGMMGLIGIKLSAVPVVILIASVGIGVEFTVHVALAFLTAIGDKNRRAVLAL
MPTC    LALMTVELFGMMGLIGIKLSAVPVVILIASVGIGVEFTVHVALAFLTAIGDKNHRAMLAL
PTC     VLASLAQIFGAMTLLGIKLSAIPAVILILSVGMMLCFNVLISLGFMTSVGNRQRRVQLSM
BPTC    LATLVLQLLGVMALLGVKLSAMPPVLLVLAIGRGVHFTVHLCLGFVTSIGCKRRRASLAL

HPTC    EHMFAPVLDGAVSTLLGVLMLAGSEFDFIVRYFFAVLAILTILGVLNGLVLLPVLLSFFG
MPTC    EHMFAPVLDGAVSTLLGVLMLAGSEFDFIVRYFFAVLAILTVLGVLNGLVLLPVLLSFFG
PTC     QMSLGPLVHGMLTSGVAVFMLSTSPFEFVIPHFCWLLLVVLCVGACNSLLVFPILLSMVG
BPTC    ESVLAPVVHGALAAALAASMLA.ASEFGFVARLFLRLLLALVFLGLIDGLLFFPIVLSILO

HPTC    PYPEVSPANGLNRLPTPSPEPPPSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSE-EL
MPTC    PCPEVSPANGLNRLPTPSPEPPPSVVRFAVPPGHTNNGSDSSDSEYSSQTTVSGISE-EL
PTC     PEAELVPLEHPDRISTPSPLPVRSSKRSGKSYVVQGSRSSRGSCQKSHHHHHKDLNDPSL
BPTC    PAAEVRPIEHPERLSTPSPKCSPIHPRKSSSSSGGGDKSSRTS--KSAPRPC----APSL

HPTC    RHYEAQQGAGGPAHQVIVEATENPVFAHSTVVHPESRHHPPSNPRQQPHLDSGSLPPGRQ
MPTC    RQYEAQQGAGGPAHQVIVEATENPVFARSTVVHPDSPHQPPLTPRQQPHLDSGSLSPGRQ
PTC     TTITEEPQSWKSSNSSIQMPNDWTYQPREQ--RPASYAAPPPAYHKAAAQQHHQHQGPPT
BPTC    TTITEEPSSWHSSAHSVQSSMQSIVVQPEVVVETTTYNGSDSASGRSTPTKSSHGGAITT

HPTC    GQQPRRDPPREGLWPPLYRPRRDAFEISTEGHSGPSNRARWGPRGARSHNPPNPASTAMG
MPTC    GQQPRRDPPREGLRPPPYRPRRDAFEISTEGHSGPSNRDRSGPRGARSHNPRNPTSTAMG
PTC     TPPPPFPTA----------YPPELQSIVVQPEVTVETTHS----------DS
BPTC    TKVTATANIKVEVVTPSDRKSRRSYHYYDRRRDRDEDRDRDRERDRDRDRDRDRDRDRDR

HPTC    SSVPGYCQPITTVTASASVTVAVHPPPVPGPGRNPRGGLCPGY---PETDHGLFEDPHVP
MPTC    SSVPSYCQPITTVTASASVTVAVHPP--PGPGRNPRGGPCPGYESYPETDHGVFEDPHVP
PTC     NT--------TKVTATANIKVELAMP-----GPAVRS---YNFTS----------
BPTC    DR--------DRERSRERDRP.DRYRD-----EPDHPA---SPRENGRDSGHE-------

HPTC    FHVRCERRDSKVEVIELQDVECEERPRGSSSN
MPTC    FHVRCERRDSKVEVIELQDVECEERPWGSSSN
PTC     ----------
BPTC    ---------- SDSSRH
```

The identity of ten other clones recovered from the mouse library is not determined.

These cDNAs cross-hybridize with mouse *ptc* sequence, while differing as to their restriction

maps. These genes encode a family of proteins related to the patched protein. Alignment of the human and mouse nucleotide sequences, which includes coding and noncoding sequence, reveals 89% identity.

Radiation hybrid mapping of the human ptc gene. Oligonucleotide primers and conditions for specifically amplifying a portion of the human *ptc* gene from genomic DNA by the polymerase chain reaction were developed. This marker was designated STS SHGC-8725. It generates an amplification product of 196 bp, which is observed by agarose gel electrophoresis when o human DNA is used as a template, but not when rodent DNA is used. Samples were scored in duplicate for the presence or absence of the 196 bp product in 83 radiation hybrid DNA samples from the Stanford G3 Radiation Hybrid Panel (purchased from Research Genetics, Inc.) By comparison of the pattern of G3 panel scores for those with a series of Genethon meiotic linkage 5 markers, it was determined that the human *ptc* gene had a two point lod score of 1,000 with the meiotic marker D9S287, based on no radiation breaks being observed between the gene and the marker in 83 hybrid cell lines. These results indicate that the *ptc* gene lies within 50-100 kb of the marker. Subsequent physical mapping in YAC and BAC clones confirmed this close linkage estimate. Detailed map information can be obtained from http://www.shgc.stanford.edu.

Analysis of BCNS mutations. The basal cell nevus syndrome has been mapped to the same region of chromosome 9q as was found for *ptc*. An initial screen of EcoRl digested DNA from probands of 84 BCNS kindreds did not reveal major rearrangements of the *ptc* gene, and so screening was performed for more subtle sequence abnormalities. Using vectorette PCR, by the method according to Riley *et al.* (1990) N.A.R. 18:2887-2890, on a BAC that contains genomic DNA for the entire coding region of *ptc*, the intronic sequence flanking 20 of the 24 exons was determined. Single strand conformational polymorphism analysis of PCR-amplified

DNA from normal individuals, BCNS o patients and sporadic basal cell carcinomas (BCC) was performed for 20 exons of *ptc* coding sequence. The amplified samples giving abnormal bands on SSCP were then sequenced.

In blood cell DNA from BCNS individuals, four independent sequence changes were found; two in exon 15 and two in exon 1 0. One 49 year old man was found to have a sequence change in exon 15. His affected sister and daughter have the same alteration, but three unafflicted relatives do not. His blood cell DNA has an insertion of 9 base pairs at nucleotide 2445 of the coding sequence, resulting in the insertion of three amino acids (PNI) after amino acid 815. Because the normal sequence preceding the insertion is also PNI, a direct repeat has been formed.

The second case of an exon 15 change is an 18 year old woman who developed jaw cysts at age 9 and BCCs at age 6. The developmental effects together with the BCCs indicate that she has BCNS, although none of her relatives are known to have the syndrome. Her blood cell DNA has a deletion of 11 bp, removing the sequence ATATCCAGCAC at nucleotides 2441 to 2452 of the coding sequence. In addition, nucleotide 2452 is changed from a T to an A. The deletion results in a frameshift that is predicted to truncate the protein after amino acid 813 with the addition of 9 amino acids. The predicted mutant protein is truncated after the seventh transmembrane domain. In *Drosophila, a ptc* protein that is truncated after the sixth transmembrane domain is inactive when ectopically expressed, in contrast to the full-length protein, suggesting that the human protein is inactivated by the exon 15 sequence change. The patient with this mutation is the first affected family member, since her parents, age 48 and 50, have neither BCCs nor other signs of the BCNS- DNA from both parents' genes have the normal nucleotide sequence for exon 15, indicating that the alteration in exon 15 arose in the same generation as did the BCNS phenotype. Hence her disease is the result of a new mutation. This

sequence change is not detected in 84 control chromosomes.

Analysis of sporadic basal cell carcinomas. To determine whether *ptc* is also involved in BCCs that are not associated with the BCNS or germline changes, DNA was examined from 12 sporadic BCCS. Three alterations were found in these tumors. In one tumor, a C to T transition in exon 3 at nucleotide 523 of the coding sequence changes a highly conserved leucine to phenylalanine at residue 175 in the first putative extracellular loop domain Blood cell DNA from the same individual does not have the alteration, suggesting that it arose somatically in the tumor. SSCP was used to examine exon 3 DNA from 60 individuals who do not have BCNS, and found no changes from the normal sequence. Two other sporadic BCCs have deletions o encompassing exon 9 but not extending to exon 8.

The existence of sporadic and hereditary forms of BCCs is reminiscent of the characteristics of the two forms of retinoblastoma. This parallel, and the frequent deletion in tumors of the copy of chromosome 9q predicted by linkage to carry the wild-type allele, demonstrates that the human *ptc* is a tumor suppressor gene. *ptc* represses a variety of genes, including growth factors, during Drosophila development and may have the same effect in human skin. The often reported large body size of BCNS patients also could be due to reduced *ptc* function, perhaps due to loss of control of growth factors. The C to T transition identified in *ptc* in the sporadic BCC is also a common genetic change in the *p53* gene in BCC and is consistent with the role of sunlight in causing these tumors. By contrast, the inherited deletion and insertion mutations identified in BCNS patients, as expected, are not those characteristic of ultraviolet mutagenesis.

The identification of the *ptc* mutations as a cause of BCNS links a large body of developmental genetic information to this important human disease. In embryos lacking *ptc* function part of each body segment is transformed into an anterior-posterior mirror-image

duplication of another part. The patterning changes in *ptc* mutants are due in part to derepression of another segment polarity gene, *wingless*, a homolog of the vertebrate Wnt genes that encodes secreted signaling proteins. In normal embryonic development, *ptc* repression of *wg* is relieved by the Hh signaling protein, which emanates from adjacent cells in the posterior part of each segment. The resulting localized wg expression in each segment primordium organizes the pattern of bristles on the surface of the animal. The *ptc* gene inactivates its own transcription, while Hh signaling induces *ptc* transcription.

In flies two other proteins work together with Hh to activate target genes: the ser/thr kinase *fused* and the zinc finger protein encoded by *cubitus interruptus*. Negative regulators working together with *ptc* to repress targets are *protein kinase A and costal2*. Thus, mutations that inactivate human versions of *protein kinase A or costal2*, or that cause excessive activity of human *hh*, *gli*, or a *fused* homolog, may modify the BCNS phenotype and be important in tumorigenesis.

In accordance with the subject invention, mammalian patched genes, including the mouse and human genes, are provided, which can serve many purposes. Mutations in the gene are found in patients with basal cell nevus syndrome, and in sporadic basal cell carcinomas. The autosomal dominant inheritance of BCNS indicates that *patched* is a tumor suppressor gene. The patched protein may be used in a screening for agonists and antagonists, and for assaying for the transcription of *ptc* mRNA. The protein or fragments thereof may be used to produce antibodies specific for the protein or specific epitopes of the protein. In addition, the gene may be employed for investigating embryonic development, by screening fetal tissue, preparing transgenic animals to serve as models, and the like.

As described above, patients with basal cell nevus syndrome have a high incidence of multiple basal cell carcinomas, medulloblastomas, and meningiomas. Because somatic *ptc*

mutations have been found in sporadic basal cell carcinomas, we have screened for *ptc* mutations in several types of sporadic extracutaneous tumors. We found that 2 of 14 sporadic medulloblastomas bear somatic nonsense mutations in one copy of the gene and also deletion of the other copy. In addition, we identified mis-sense mutations in *ptc* in two of seven breast carcinomas, one of nine meningiomas, and one colon cancer cell line. No *ptc* gene mutations were detected in 10 primary colon carcinomas and eighteen bladder carcinomas.

BCNS[3] (OMIM #109400) is a rare autosomal dominant disease with diverse phenotypic abnormalities, both tumorous (BCCs, medulloblastomas, and meningiomas) and developmental (misshapen ribs, spina bifida occults, and skull abnormalities; Gorlin, R.J.(1987) *Medicine* 66:98-113). The BCNS gene was mapped to chromosome 9q22.3 by linkage analysis of BCNS families and by LOH analysis in sporadic BCCs (Gallani, M.R. *et al.* (1992) *Cell* 69:111-117). LOH in sporadic medulloblastomas has been reported in the same chromosome region (Schofield, D. *et al.* (1995) *Am J Pathol* 146:472-480). Recently, the human homologue of the *Drosophila patched* (PTCH) gene has been mapped to the BCNS region (Hahn, H. *et al.* (1996) *Cell* 85:841-851; Johnson, R.L. *et al.* (1996) *Science* 272:1668-1671; Gallani, M.R. *et al.* (1996) *Nat Genet* 14:78-81; Xie, J. *et al.* (1997) *Genes Chromosomes Cancer* 18:305-309), and mutations in this gene have been found in the blood DNA of BCNS patients and in the DNA of sporadic BCCs (Hahn, H. *et al., supra;* Johnson, R.L. *et al., supra;* Gallani, M.R. *et al., supra;* and Chidambaram, A. *et al.* (1996) *Cancer Res* 36:4599-4601). *ptc* appears to function as a tumor suppressor gene; inactivation abrogates its normal inhibition of the hedgehog signaling pathway. Because of the wide variety of tumors in patents with the BCNS and wide tissue distribution of *ptc* gene expression, we have begun screening for *ptc* gene mutations in several types of human cancers, especially those present in increased numbers in BCNS patients (medulloblastomas), those in tissues derived embryologically from epidermis (breast carcinomas)

and those with chromosome 9q LOG (bladder carcinomas; see Cairns, P. *et al.* (1993) *Cancer Res* 53:1230-1232; and Sidransky, D. *et al.* (1997) *NEJM* 326:737-740).

Materials and methods

Clinical Materials. Diagnoses of all tumors were confirmed histologically. Cell lines were obtained from the America Type Culture Collection. DNA was extracted from tumors or matched normal tissue (peripheral blood leukocytes or skin) as described (Cogen, P.H. *et al.* (1990) *Genomics* 8:279-285; and Sambrook, J. *et al.* Molecular Cloning: A Laboratory Manual, Ed. 2, Vol. 2, pp. 9.17 - 9.19, Cold Spring Harbor, NY (1989)).

PCR and Heteroduplex Analysis. PCR amplification and heteroduplex/SSCP analysis were performed as described (Johnson, R.L. *et al.*, *supra*; Spritz, R.A. *et al.* (1992) *Am J Hum Genet* 51:1058-1065). Primers used and intron/exon boundary sequences of the *ptc* gene were derived as reported previously (Johnson, R.L. *et al.*, *supra*) and are shown in Table 1. Primers for exon 1 and 2 were from Hahn *et al.* (*supra*).

Sequence Analysis. Exon segments exhibiting bands were reamplified and were sequenced directly using the Sequenase sequencing kit according to the protocol recommended by the manufacturer (United States Biochemical Corp.). A second sequencing was performed using independently amplified PCR products to confirm the sequence change. The amplified PCR products from each tumor were also cloned into the plasmid vector pCR 2.1 (InVitrogen), followed by sequence analysis of at least four independent clones. The sequence alteration was confirmed from at least two independent clones. Simplified amplification of specific allele analysis was performed according to Lei and Hall (Lei, X. and Hall, B.G. (1994) *Biotechniques* 16:44-45).

Allele Loss Analysis. Microsatellites used for allelic loss analysis were D9S109, DpS119, D9S127, D9S196, and D9S287 described in the CHLC human screening set (Research

Genetics). A part of the *ptc* intron 1 sequence was tested for polymorphism in a control population and found to be polymorphic in 80% of the samples tested. This microsatellite was used for analysis of *ptc* gene allelic loss in bladder carcinomas. The primer sequences are as follows: forward primer, 5'-CTGAGCAGATTTCCCAGGTC-3'; and reverse primer, 5'-CCTCAGACAGACCTTTCCTC-3'. The PCR cycling for this newly isolated marker was 4 min. at 95°C, followed by 30 cycles of 40 s at 95°C, 2 min. at 60°C, and 1 min. at 72°C. PCR products were separated on 6% polyacrylamide gels and exposed to film.

Results and Discussion

Intronic boundaries were determined for 22 exons of *ptc* by sequencing vectorette PCR products derived from BAC 192J22 (Johnson R.L., *supra*; Table 1). Our findings are in agreement with those of Hahn *et al.* (*supra*), expect that we find exon 12 is composed of 2 separate exons of 126 and 119 nucleotides. This indicates that *ptc* is composed of 23 coding exons instead of 22. In addition, we find that exons 3, 4, 10, 11, 17, 21, and 23 differ slightly in size than reported previously (Hahn *et al.*, *supra*). Of 63 tumors studied, 14 were sporadic medulloblastomas, and 9 were sporadic meningiomas. These 23 tumors were examined for allelic deletions by genotyping of tumor and blood DNA with microsatellite markers that flank the *ptc* gene: D9S119, D9S196, D9S287, D9S127, and D9S109. Four of 14 medulloblastomas had LOH. Two of the medulloblastomas, both of which had LOH, had mutations (med34 and med36; see Cogen, P.H. *et al.*, *supra*), which are predicted to result in truncated proteins (Table 2). DNA samples from the blood of these patients lack these mutations, indicating that they both are somatic mutations. med34 also has allelic loss on 17p (Cogen, P.H. *et al.*, *supra*). We were unable to detect *ptc* gene mutations by heteroduplex analysis in the other two medulloblastomas bearing LOH on 9q. The pathological features of these two tumors differed in that med34 belongs t the desmoplastic subtype, whereas med36 is of the classic type,

5 indicating that *ptc* mutations in medulloblastomas are not restricted to a specific subtype.

TABLE 1 *Primers and boundary sequences of PTCH*

	5' Boundary[a]	Nucleotide position[b]	Exon size	3' boundary[a]	Reading frame[c]	Primers
1	ND[d]	ND	ND	agtGTGNAT	ND	
2	ND	202	189	agtGTAAGA	3	
3	cTCAGt	335	190	cagGTAAGG	1	3F GAGTTTGCAGTGATGTTGCTNTTC 3R ACCGCCTTACCTGCTGCTC
4	TATTAGt	555	70	cagGTATAT	2	4F TGCACTAATTTTCTTATTACACTC 4R TAAGGCACACTACTGGGGTG
5	TGACAGt	655	92	ccaGTAAGT	3	5F GAACACCCCAGTAGTGTGCC 5R TGAGTCCTAGAGAAGTCACAGACA
6	TTGCACt	747	199	aaaGTGAGT	2	6F GGCTCTTTTCATGTTCTCGTC 6R TGTTTTGCTCTCCACCCTTC
7	TTTTAGt	946	122	cagGTAAGC	3	7F GCACTGGATTTTAACAAGGCATG 7R AGGGCATAGATTGTCCCCGG
8	CTGCAGt	1068	148	gagGTAAAC	2	8F TGGGAATACTGATCATGTGCC 8R CATAACCACCGATTCTGCAC
9	CCACAGt	1216	132	acgGTAACG	3	9F CATTTGGGCATTTCCCATTC 9R ACCAAACCAAACTCCAGCCC
10	TTGCAGt	1348	156	cagGTACTA	3	10F TGCCCCCATTGTTCTGCTTG 10R GGACAGCAGATAAACGGCTCC
11	CTGTAGt	1504	99	gagGTAATG	3	11F GCATCTCCCATGTCTAATGCCAC 11R AAGCTGTGATGTCCCCAAAG
12	TCCCAGt	1603	126	cagGTGAGC	3	12F GACCATGTCCACTTCAGTTC 12R CGTTCAGGATCACTACAGCC
13	TCCCAGt	1729	119	aagGTACAT	3	13F AGTCCTCTGATTGGCGGAG 13R CCATTCTGCACCCAATCAAAAC
14	TTTCAGt	1848	403	aagGTAATC	2	14F AAAATGGCAGAATGAAGGACC 14R CTGATGAACTCCCAAATCTTCTG
15	TTCCAGt	2251	310	aggGTAAGA	3	15F GGAAGAGTCAGTGGTGCTCC 15R CGCCAAAGACCGAAAGGAC
16	TTCTAGt	2561	143	cagGTACTC	1	16F AGGCTCCTTCTGCCTGCGAG 16R GCTGTCAAGCAGCCTCCAC
17	TTGTAGt	2704	184	gaaGTAAGT	3	17F GCTCTCAAGGCAGAAGTCCG 17R GGAAGGCACCTCTCTTAAGTAC
18	GTCCAGt	2888	281	aatGTGAGT	1	18F GCTCCTAACCTGTGTCCTCTTC 18R GAATTTGACTTCCACAAAGCCC
19	CTCCAGt	3169	138	agGTATGG	3	19F CGCCCACTGACCAGTGTGTG 19R GAGCCAGAGGAAATGGTGTG
20	GCACAGt	3307	143	cagGTAAGC	3	20F AGCATTTACCAGTGGAAGTCC 20R TTGCACACGCCTGCTTAC
21	TCCCAGt	3450	100	gagGTCAGT	2	21F TGTTCCCGTTTCCTCTTG 21R GCACAGGAAACACACCATTC
22	AAATAGt	3550	255	actGTAAGT	3	22F GCAGGTAAATGGACAATGACAC 22R ACTACCACGGTGGGACGACC
23	CTGCAGt	3805	541	gagGTGAGT	3	23F CCCTTCTAACCCACCCTCAC 23R GACACATCAGCCTTGCTC
24	ND	4346	ND	ND		

[a] Consensus sequences for the 5' and 3' exonic boundaries are $(T_C)_2NCAG|g$ and $ag|GT^G_AAGT$, respectively (20). Lower case denotes exonic sequence.
[b] Exon positions are in reference to the coding sequence of *PTCH* (3) with the beginning ATG as nucleotide 1.
[c] Exon boundary begins after the first, second, or third base of the codon of the translational reading frame.
[d] ND, not determined.

One report (Schofield, D. *et al., supra*) has shown that five medulloblastomas (two 25 BCNS-associated cases and three sporadic cases) bearing LOH on chromosome 9q22.3-q31 are all of the desmoplastic subtype, suggesting LOH on 9q22.3 is histological subtype specific. We feel that the conclusion derived from only five positive tumors is a not strong one because we and others (Raffel, C. *et al.* (1997) *Cancer Res* 57:842-845) have found nondesmoplastic

subtypes of medulloblastomas bearing LOH on chromosome 9q22.3. Independently, another group has reported their finding of *ptc* mutations in sporadic medulloblastomas (Raffel, C. *et al, supra*).

A change of T to C at nucleotide 2990 (in exon 18) was identified in DNA from one of nine sporadic meningiomas, causing a predicted change of codon 997 from Ile to Thr (Table 2). The meningioma bearing this mutation also has allelic loss on 9q22.3. Blood cell DNA is heterozygous for this mutation, but DNA from the tumor contains only the mutant sequence. Of 100 normal chromosomes examined, none has this sequence change, suggesting that this mutation is not likely a common polymorphism. This patient is 84 years old and has had no phenotypic abnormalities suggestive of the BCNS, suggesting that this sequence alteration may not have caused complete inactivation of the *ptc* gene. None of the other eight meningiomas had detectable LOH at chromosome 9q.

TABLE 2 *PATCHED gene alterations*[a]

Tumor	Pathology	Nucleotide	Codon	Exon	Consequence	LOH	Mutation Type
Med34	Medulloblastoma (desmoplastic)	TC1869A	623	14	Frameshift	Yes	Somatic
Med36	Medulloblastoma (classic)	G2503T	835	15	Glu to STOP	Yes	Somatic
Men1	Meningioma	T2990C	997	18	Ile to Thr	Yes	Germ-line
Br349	Breast carcinoma	T2863C	955	17	Tyr to His	Yes	Somatic
Br321	Breast carcinoma	A2975G	995	18	Glu to Gly	No	Somatic
Co320	Colon tumor cell line	A2000C	667	14	Glu to Ala	No	Unknown
Co8-1	Colon carcinoma	T to C	Intron 10		Polymorphism	No	Germ-line
Co15-1	Colon carcinoma	T to C	Intron 10		Polymorphism	No	Germ-line

We also examined a variety of other tumors (10 primary tumors and 1 cell line), 18 bladder tumors (14 primary tumors and 4 cell lines), and 2 ovarian cancer cell lines. These tumors are not known to occur in higher than expected frequency in BCNS patients. We identified sequence abnormalities in two breast carcinomas and in the one colon cancer cell line (Table 2). The mutation found in breast carcinoma Br349 is not present in the patient's normal

skin DNA, indicating that the sequence change is a somatic mutation. Direct sequencing of the PCR product indicated that only the mutant allele is present in the tumor. This mutation changes codon 955 from Tyr to His, and this Tyr is conserved in human, murine, chicken, and fly *ptc*II homologues (Goodrich, L.V. *et al.* (1996) *Genes Dev* 10:301-312). The mutation in breast carcinoma Br321 is predicted to change codon 995 from Glu to Gly, and the tumor with this mutation retains the wild-type allele. We have sequenced exon 18 in DNA from the blood of 50 normal person s and found no changes from the published sequence, suggesting that the sequence change found in Br321 is not a common polymorphism. Furthermore, examination of the DNA from the cultured skin fibroblasts of the patient did not reveal the same mutation, indicating that this is a somatic mutation.

Because DNA is not available from normal cells of the patient from which colon cell line 320 was established, we used simplified amplification of specific allele analysis (Lei, X. and Hall, B.G., *supra*) to examine 50 normal blood DNA samples for the presence of the sequence alteration and found none but the DNA from this cell line to have the mutant allele, suggesting that this mutation also is unlikely to be a common sequence polymorphism. For bladder carcinomas, a newly isolated microsatellite that was derived from intron 1 of the *ptc* gene was used to examine LOH in the tumor. Three primary bladder carcinomas showed LOH at this intragenic locus. With no *ptc* mutations detected in these tumors, we suspect that the LOH in these three bladder carcinomas may reflect the high incidence of while chromosome 9 loss in bladder cancers (Sidransky, D. *et al.*, *supra*). A similar observation has been reported previously (Simoneau, A. R. *et al.* (1996) *Cancer Res* 56:5039-5043).

We also detected a sequence change in intron 10 in two colon carcinomas, 15-1 and 8-1, an alteration that was reported previously as a splicing mutation (Unden, A.B. *et al.* (1996) *Cancer Res* 56:4562-4565). Because we found the same sequence change in about 20% of

normal control samples, we suggest that this more likely is a nonpathogenic polymorphism. The *ptc* protein is predicted to contain 12 transmembrane domains, two large extracellular loops, and one intracellular loop (Goodrich, L.V. *et al., supra*). Of the six mutations we identified, four are missense mutations. Three mutations lead to amino acid substitutions in the second extracellular loop, and one mutation results in an amino acid change in the intracellular domain.

Our data indicate that somatic inactivation of the *ptc* gene does occur in some sporadic medulloblastomas. In addition, because missense mutations of the *ptc* gene were detected in breast carcinomas, we suspect that defects of the *ptc* function also may be involved in some breast carcinomas, although biochemical evidence is necessary to show how these missense mutations might impair *ptc* function. Of 11 colon cancers and 18 bladder carcinomas examined, we found only one mutation in 1 colon cell line, suggesting that *ptc* gene mutations are relatively uncommon in clon and bladder cancers, although the incidence of chromosome 9 loss in bladder cancers is high (Cairns, P. *et al., supra*).

Published reports of SSCP analysis of tumor DNA identified mutations in the *ptc* gene in only 30% of sporadic BCCs, although chromosome 9q22.3 LOH was reported in more than 50% of these tumors (Gallani, M.R. *et al., supra*). It has been reported that heteroduplex/SSCP analysis of gene mutations is more sensitive than SSCP analysis (Spritz, R.A. *et al., supra*). In our studies, we were able to identify a point mutation in the 310-bp PCR product from exon 15 using heteroduplex analysis, whereas SSCP analysis failed to reveal this sequence change (Table 2). Therefore, we suspect that there may be more mutations in BCCs than we have found thus far. Analysis of the *ptc* gene in BCNS patients and in sporadic BCCs has identified mutations scattered widely across the gene, and the majority of mutations were predicted to result in truncated proteins (Hahn, H. *et al., supra*; Johnson, R.L. *et al., supra*; Gallani, M.R. *et al., supra*; Chidambaram, A. *et al., supra*; Unden, A.B. *et al., supra*; Wicking, C. *et al.* (1997) *Am*

J Hum Genet 60:21-26). In our screening, we found two breast carcinomas bearing missense mutations of the *ptc* gene. In one of these two tumors, B349, direct sequencing indicated a deletion of the other copy of the *ptc* gene. Any comparison of mutations in skin cancers versus extracutaneous tumors must consider the wholly different causes of these mutations; UV light is unique to the skin.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent o application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SCOTT, MATTHEW P.
GOODRICH, LISA V.
JOHNSON, RONALD L.

(ii) TITLE OF INVENTION: Patched Genes and Their Use

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Foley, Hoag & Eliot LLP
(B) STREET: One Post Office Square
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: US
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Vincent, Matthew P.
(B) REGISTRATION NUMBER: 36,709
(C) REFERENCE/DOCKET NUMBER: SUV003.26

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-832-1000
(B) TELEFAX: 617-832-7000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 736 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACNNCNNTN NATGGCACCC CCNCCCAACC TTTNNNCCNN NTAANCAAAA NNCCCCNTTT 60

NATACCCCCT NTAANANTTT TCCACCNNNC NNAAANNCCN CTGNANACNA NGNAAANCCN 120

TTTTTNAACC CCCCCCACCC GGAATTCCNA NTNNCCNCCC CCAAATTACA ACTCCAGNCC 180

AAAATTNANA NAATTGGTCC TAACCTAACC NATNGTTGTT ACGGTTTCCC CCCCCAAATA 240

CATGCACTGG CCCGAACACT TGATCGTTGC CGTTCCAATA AGAATAAATC TGGTCATATT 300

AAACAAGCCN AAAGCTTTAC AAACTGTTGT ACAATTAATG GGCGAACACG AACTGTTCGA 360

ATTCTGGTCT GGACATTACA AAGTGCACCA CATCGGATGG AACCAGGAGA AGGCCACAAC 420

CGTACTGAAC GCCTGGCAGA AGAAGTTCGC ACAGGTTGGT GGTTGGCGCA AGGAGTAGAG 480

TGAATGGTGG TAATTTTTGG TTGTTCCAGG AGGTGGATCG TCTGACGAAG AGCAAGAAGT 540

CGTCGAATTA CATCTTCGTG ACGTTCTCCA CCGCCAATTT GAACAAGATG TTGAAGGAGG 600

CGTCGAANAC GGACGTGGTG AAGCTGGGGG TGGTGCTGGG GGTGGCGGCG GTGTACGGGT 660

GGGTGGCCCA GTCGGGGCTG GCTGCCTTGG GAGTGCTGGT CTTNGCGNGC TNCNATTCGC 720

CCTATAGTNA GNCGTA 736

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Pro Pro Pro Asn Tyr Asn Ser Xaa Pro Lys Xaa Xaa Xaa Leu Val
1 5 10 15

Leu Thr Pro Xaa Val Val Thr Val Ser Pro Pro Lys Tyr Met His Trp
20 25 30

Pro Glu His Leu Ile Val Ala Val Pro Ile Arg Ile Asn Leu Val Ile
35 40 45

Leu Asn Lys Pro Lys Ala Leu Gln Thr Val Val Gln Leu Met Gly Glu
50 55 60

His Glu Leu Phe Glu Phe Trp Ser Gly His Tyr Lys Val His His Ile
65 70 75 80

Gly Trp Asn Gln Glu Lys Ala Thr Thr Val Leu Asn Ala Trp Gln Lys
85 90 95

Lys Phe Ala Gln Val Gly Gly Trp Arg Lys Glu
100 105

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5187 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC 60

CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCC 120

GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC 180

GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG 240

GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG 300

TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAAA AGAACTGCGG CAAGTTTTTG 360

GTTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG 420

ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT 480

ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA 540

AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGACTCAGCA 600

CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTTG 660

TGCTACAAAT CAGGGGAACT TATCACGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC 720

CTTTACCCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GGGAAGGGGC AAAGCTACAG 780

TCCGGGACAG CATACCTCCT AGGTAAGCCT CCTTTACGGT GGACAAACTT TGACCCCTTG 840

GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG 900

AATAAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA 960

GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT 1020

TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT 1080

GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAACTTGTCA GCGCTCACGC CCTGCAAACC 1140

ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC 1200

TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT 1260
TACGTGGAGG TGGTTCATCA AAGTGTCGCC CCAAACTCCA CTCAAAAGGT GCTTCCCTTC 1320

ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG 1380

GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC 1440

TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GGCGTCCTGT TGGTTGCGCT GTCAGTGGCT 1500

GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT 1560

TTGCCGTTTC TTGCTCTTGG TGTTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC 1620

AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGACTGGGGA GTGCCTCAAG 1680

CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTCATGGCC 1740

GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA 1800

TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA 1860

CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG 1920

ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCCA 1980

CCCCCATACA CCAGCCACAG CTTCGCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT 2040

CAGCTCCGCA CAGAGTATGA CCCTCACACG CACGTGTACT ACACCACCGC CGAGCCACGC 2100

TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC 2160

GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC 2220

CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT 2280

TTCCTCCTGA AACCCAAAGC CAAGGTTGTG GTAATCCTTC TTTTCCTGGG CTTGCTGGGG 2340

GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC 2400

CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC 2460

ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT 2520

CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA 2580

ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC 2640

TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCCTC 2700

GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG 2760

ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAATC CGAGCGCTTT CTACATCTAC 2820

CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG 2880

CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA 2940

ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA 3000

GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG 3060

AGCCTGGGAC TGTCCAGCTA CCCCAATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC 3120

AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTTCTAGTG 3180

TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG 3240

ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT 3300

GTGGTCATCC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG 3360

GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG 3420

TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG 3480

TCCGAATTTG ATTTCATTGT CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG 3540

GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCCT 3600

GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCACTC CTTCGCCTGA GCCGCCTCCA 3660

AGTGTCGTCC GGTTTGCCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC 3720

TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA 3780

GCACAGCAGG GTGCCGGAGG CCCTGCCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCCT 3840

GTCTTTGCCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT 3900

CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT 3960

CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCCCT ACAGACCGCG CAGAGACGCT 4020

TTTGAAATTT CTACTGAAGG GCATTCTGGC CCTAGCAATA GGGACCGCTC AGGGCCCCGT 4080

GGGGCCCGTT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC 4140

AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT 4200

CCCCCGCCTG GACCTGGGCG CAACCCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC 4260

CCTGAGACTG ATCACGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG 4320

AGGAGGGACT CAAAGGTGGA GGTCATAGAG CTACAGGACG TGGAATGTGA GGAGAGGCCG 4380

TGGGGGAGCA GCTCCAACTG AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA 4440

AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGGA ATTATGGGAA 4500

GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKKGTG AAATATTTCT ATAAATATTT 4560

AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTCC TGGGGCCTCT 4620

CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC 4680

TGTGCCACAA CCAAGCTTAA CTTAGTTTTA AAAAAAATCT CCCAGCATAT GTCGCTGCTG 4740

CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAAA TATTGCTTAT GTAATAGGAT 4800

TATTTGTAAA GGTTTCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860

ATGAATTGTT ACTGTTAACT TTTGAACACG CTATGCGTGG TAATTGTTTA ACGAGCAGAC 4920

ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTCGCATG 4980

GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040

TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100

GTGGGCTGGG AAGGTCCAGG TCTTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160

CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1311 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Val Ala Pro Asp Ser Glu Ala Pro Ser Asn Pro Arg Ile Thr Ala
1               5                  10                  15

Ala His Glu Ser Pro Cys Ala Thr Glu Ala Arg His Ser Ala Asp Leu
            20                  25                  30

Tyr Ile Arg Thr Ser Trp Val Asp Ala Ala Leu Ala Leu Ser Glu Leu
        35                  40                  45

Glu Lys Gly Asn Ile Glu Gly Gly Arg Thr Ser Leu Trp Ile Arg Ala
    50                  55                  60

Trp Leu Gln Glu Gln Leu Phe Ile Leu Gly Cys Phe Leu Gln Gly Asp
65                  70                  75                  80

Ala Gly Lys Val Leu Phe Val Ala Ile Leu Val Leu Ser Thr Phe Cys
                85                  90                  95

Val Gly Leu Lys Ser Ala Gln Ile His Thr Arg Val Asp Gln Leu Trp
            100                 105                 110

Val Gln Glu Gly Gly Arg Leu Glu Ala Glu Leu Lys Tyr Thr Ala Gln
        115                 120                 125

Ala Leu Gly Glu Ala Asp Ser Ser Thr His Gln Leu Val Ile Gln Thr
    130                 135                 140

Ala Lys Asp Pro Asp Val Ser Leu Leu His Pro Gly Ala Leu Leu Glu
145                 150                 155                 160

His Leu Lys Val Val His Ala Ala Thr Arg Val Thr Val His Met Tyr
                165                 170                 175

Asp Ile Glu Trp Arg Leu Lys Asp Leu Cys Tyr Ser Pro Ser Ile Pro
            180                 185                 190

Asp Phe Glu Gly Tyr His His Ile Glu Ser Ile Ile Asp Asn Val Ile
        195                 200                 205

Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ser Lys
    210                 215                 220

Leu Leu Gly Pro Asp Tyr Pro Ile Tyr Val Pro His Leu Lys His Lys
225                 230                 235                 240

Leu Gln Trp Thr His Leu Asn Pro Leu Glu Val Val Glu Glu Val Lys
                245                 250                 255

Lys Leu Lys Phe Gln Phe Pro Leu Ser Thr Ile Glu Ala Tyr Met Lys
            260                 265                 270

Arg Ala Gly Ile Thr Ser Ala Tyr Met Lys Lys Pro Cys Leu Asp Pro
        275                 280                 285

Thr Asp Pro His Cys Pro Ala Thr Ala Pro Asn Lys Lys Ser Gly His
    290                 295                 300
```

Ile Pro Asp Val Ala Ala Glu Leu Ser His Gly Cys Tyr Gly Phe Ala
305 310 315 320

Ala Ala Tyr Met His Trp Pro Glu Gln Leu Ile Val Gly Gly Ala Thr
325 330 335

Arg Asn Ser Thr Ser Ala Leu Arg Lys Ala Arg Xaa Leu Gln Thr Val
340 345 350

Val Gln Leu Met Gly Glu Arg Glu Met Tyr Glu Tyr Trp Ala Asp His
355 360 365

Tyr Lys Val His Gln Ile Gly Trp Asn Gln Glu Lys Ala Ala Ala Val
370 375 380

Leu Asp Ala Trp Gln Arg Lys Phe Ala Ala Glu Val Arg Lys Ile Thr
385 390 395 400

Thr Ser Gly Ser Val Ser Ser Ala Tyr Ser Phe Tyr Pro Phe Ser Thr
405 410 415

Ser Thr Leu Asn Asp Ile Leu Gly Lys Phe Ser Glu Val Ser Leu Lys
420 425 430

Asn Ile Ile Leu Gly Tyr Met Phe Met Leu Ile Tyr Val Ala Val Thr
435 440 445

Leu Ile Gln Trp Arg Asp Pro Ile Arg Ser Gln Ala Gly Val Gly Ile
450 455 460

Ala Gly Val Leu Leu Leu Ser Ile Thr Val Ala Ala Gly Leu Gly Phe
465 470 475 480

Cys Ala Leu Leu Gly Ile Pro Phe Asn Ala Ser Ser Thr Gln Ile Val
485 490 495

Pro Phe Leu Ala Leu Gly Leu Gly Val Gln Asp Met Phe Leu Leu Thr
500 505 510

His Thr Tyr Val Glu Gln Ala Gly Asp Val Pro Arg Glu Glu Arg Thr
515 520 525

Gly Leu Val Leu Lys Lys Ser Gly Leu Ser Val Leu Leu Ala Ser Leu
530 535 540

Cys Asn Val Met Ala Phe Leu Ala Ala Ala Leu Leu Pro Ile Pro Ala
545 550 555 560

Phe Arg Val Phe Cys Leu Gln Ala Ala Ile Leu Leu Leu Phe Asn Leu
565 570 575

Gly Ser Ile Leu Leu Val Phe Pro Ala Met Ile Ser Leu Asp Leu Arg
580 585 590

Arg Arg Ser Ala Ala Arg Ala Asp Leu Leu Cys Cys Leu Met Pro Glu
595 600 605

Ser Pro Leu Pro Lys Lys Lys Ile Pro Glu Arg Ala Lys Thr Arg Lys
610 615 620

Asn Asp Lys Thr His Arg Ile Asp Thr Thr Arg Gln Pro Leu Asp Pro
625 630 635 640

Asp Val Ser Glu Asn Val Thr Lys Thr Cys Cys Leu Ser Val Ser Leu
645 650 655

Thr Lys Trp Ala Lys Asn Gln Tyr Ala Pro Phe Ile Met Arg Pro Ala
660 665 670

Val Lys Val Thr Ser Met Leu Ala Leu Ile Ala Val Ile Leu Thr Ser
675 680 685

Val Trp Gly Ala Thr Lys Val Lys Asp Gly Leu Asp Leu Thr Asp Ile
690 695 700

Val Pro Glu Asn Thr Asp Glu His Glu Phe Leu Ser Arg Gln Glu Lys
705 710 715 720

Tyr Phe Gly Phe Tyr Asn Met Tyr Ala Val Thr Gln Gly Asn Phe Glu
725 730 735

Tyr Pro Thr Asn Gln Lys Leu Leu Tyr Glu Tyr His Asp Gln Phe Val
740 745 750

Arg Ile Pro Asn Ile Ile Lys Asn Asp Asn Gly Gly Leu Thr Lys Phe
755 760 765

Trp Leu Ser Leu Phe Arg Asp Trp Leu Leu Asp Leu Gln Val Ala Phe
770 775 780

Asp Lys Glu Val Ala Ser Gly Cys Ile Thr Gln Glu Tyr Trp Cys Lys
785 790 795 800

Asn Ala Ser Asp Glu Gly Ile Leu Ala Tyr Lys Leu Met Val Gln Thr
805 810 815

Gly His Val Asp Asn Pro Ile Asp Lys Ser Leu Ile Thr Ala Gly His
820 825 830

Arg Leu Val Asp Lys Asp Gly Ile Ile Asn Pro Lys Ala Phe Tyr Asn
835 840 845

Tyr Leu Ser Ala Trp Ala Thr Asn Asp Ala Leu Ala Tyr Gly Ala Ser
850 855 860

Gln Gly Asn Leu Lys Pro Gln Pro Gln Arg Trp Ile His Ser Pro Glu
865 870 875 880

Asp Val His Leu Glu Ile Lys Lys Ser Ser Pro Leu Ile Tyr Thr Gln
885 890 895

Leu Pro Phe Tyr Leu Ser Gly Leu Ser Asp Thr Xaa Ser Ile Lys Thr
900 905 910

Leu Ile Arg Ser Val Arg Asp Leu Cys Leu Lys Tyr Glu Ala Lys Gly
915 920 925

Leu Pro Asn Phe Pro Ser Gly Ile Pro Phe Leu Phe Trp Glu Gln Tyr
930 935 940

Leu Tyr Leu Arg Thr Ser Leu Leu Leu Ala Leu Ala Cys Ala Leu Ala
945 950 955 960

Ala Val Phe Ile Ala Val Met Val Leu Leu Leu Asn Ala Trp Ala Ala
965 970 975

Val Leu Val Thr Leu Ala Leu Ala Thr Leu Val Leu Gln Leu Leu Gly
980 985 990

Val Met Ala Leu Leu Gly Val Lys Leu Ser Ala Met Pro Ala Val Leu
995 1000 1005

Leu Val Leu Ala Ile Gly Arg Gly Val His Phe Thr Val His Leu Cys
1010 1015 1020

Leu Gly Phe Val Thr Ser Ile Gly Cys Lys Arg Arg Arg Ala Ser Leu
1025 1030 1035 1040

Ala Leu Glu Ser Val Leu Ala Pro Val Val His Gly Ala Leu Ala Ala
1045 1050 1055

Ala Leu Ala Ala Ser Met Leu Ala Ala Ser Glu Cys Gly Phe Val Ala
1060 1065 1070

Arg Leu Phe Leu Arg Leu Leu Leu Asp Ile Val Phe Leu Gly Leu Ile
1075 1080 1085

Asp Gly Leu Leu Phe Phe Pro Ile Val Leu Ser Ile Leu Gly Pro Ala
1090 1095 1100

Ala Glu Val Arg Pro Ile Glu His Pro Glu Arg Leu Ser Thr Pro Ser
1105 1110 1115 1120

Pro Lys Cys Ser Pro Ile His Pro Arg Lys Ser Ser Ser Ser Ser Gly
1125 1130 1135

Gly Gly Asp Lys Ser Ser Arg Thr Ser Lys Ser Ala Pro Arg Pro Cys
1140 1145 1150

Ala Pro Ser Leu Thr Thr Ile Thr Glu Glu Pro Ser Ser Trp His Ser
1155 1160 1165

Ser Ala His Ser Val Gln Ser Ser Met Gln Ser Ile Val Val Gln Pro
1170 1175 1180

Glu Val Val Val Glu Thr Thr Thr Tyr Asn Gly Ser Asp Ser Ala Ser
1185 1190 1195 1200

Gly Arg Ser Thr Pro Thr Lys Ser Ser His Gly Gly Ala Ile Thr Thr
1205 1210 1215

Thr Lys Val Thr Ala Thr Ala Asn Ile Lys Val Glu Val Val Thr Pro
1220 1225 1230

Ser Asp Arg Lys Ser Arg Arg Ser Tyr His Tyr Tyr Asp Arg Arg Arg
1235 1240 1245

Asp Arg Asp Glu Asp Arg Asp Arg Asp Arg Glu Arg Asp Arg Asp Arg
1250 1255 1260

Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg
1265 1270 1275 1280

Glu Arg Ser Arg Glu Arg Asp Arg Arg Asp Arg Tyr Arg Asp Glu Arg
1285 1290 1295

Asp His Arg Ala Ser Pro Arg Glu Lys Arg Gln Arg Phe Trp Thr
1300 1305 1310

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4434 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAAACAAGA GAGCGAGTGA GAGTAGGGAG AGCGTCTGTG TTGTGTGTTG AGTGTCGCCC 60

ACGCACACAG GCGCAAAACA GTGCACACAG ACGCCCGCTG GGCAAGAGAG AGTGAGAGAG 120

AGAAACAGCG GCGCGCGCTC GCCTAATGAA GTTGTTGGCC TGGCTGGCGT GCCGCATCCA 180

CGAGATACAG ATACATCTCT CATGGACCGC GACAGCCTCC CACGCGTTCC GGACACACAC 240

GGCGATGTGG TCGATGAGAA ATTATTCTCG GATCTTTACA TACGCACCAG CTGGGTGGAC 300

GTTCAAGTGG CGCTCGATCA GATAGATAAG GGCAAAGCGC GTGGCAGCCG CACGGCGATC 360

TATCTGCGAT CAGTATTCCA GTCCCACCTC GAAACCCTCG GCAGCTCCGT GCAAAAGCAC 420

GCGGGCAAGG TGCTATTCGT GGCTATCCTG GTGCTGAGCA CCTTCTGCGT CGGCCTGAAG 480

AGCGCCCAGA TCCACTCCAA GGTGCACCAG CTGTGGATCC AGGAGGGCGG CCGGCTGGAG 540

GCGGAACTGG CCTACACACA GAAGACGATC GGCGAGGACG AGTCGGCCAC GCATCAGCTG 600

CTCATTCAGA CGACCCACGA CCCGAACGCC TCCGTCCTGC ATCCGCAGGC GCTGCTTGCC 660

CACCTGGAGG TCCTGGTCAA GGCCACCGCC GTCAAGGTGC ACCTCTACGA CACCGAATGG 720

GGGCTGCGCG ACATGTGCAA CATGCCGAGC ACGCCCTCCT TCGAGGGCAT CTACTACATC 780

GAGCAGATCC TGCGCCACCT CATTCCGTGC TCGATCATCA CGCCGCTGGA CTGTTTCTGG 840

GAGGGAAGCC AGCTGTTGGG TCCGGAATCA GCGGTCGTTA TACCAGGCCT CAACCAACGA 900

CTCCTGTGGA CCACCCTGAA TCCCGCCTCT GTGATGCAGT ATATGAAACA AAAGATGTCC 960

GAGGAAAAGA TCAGCTTCGA CTTCGAGACC GTGGAGCAGT ACATGAAGCG TGCGGCCATT 1020

GGCAGTGGCT ACATGGAGAA GCCCTGCCTG AACCCACTGA ATCCCAATTG CCCGGACACG 1080

GCACCGAACA AGAACAGCAC CCAGCCGCCG GATGTGGGAG CCATCCTGTC CGGAGGCTGC 1140

TACGGTTATG CCGCGAAGCA CATGCACTGG CCGGAGGAGC TGATTGTGGG CGGACGGAAG 1200

AGGAACCGCA GCGGACACTT GAGGAAGGCC CAGGCCCTGC AGTCGGTGGT GCAGCTGATG 1260

ACCGAGAAGG AAATGTACGA CCAGTGGCAG GACAACTACA AGGTGCACCA TCTTGGATGG 1320

ACGCAGGAGA AGGCAGCGGA GGTTTTGAAC GCCTGGCAGC GCAACTTTTC GCGGGAGGTG 1380

GAACAGCTGC TACGTAAACA GTCGAGAATT GCCACCAACT ACGATATCTA CGTGTTCAGC 1440

TCGGCTGCAC TGGATGACAT CCTGGCCAAG TTCTCCCATC CCAGCGCCTT GTCCATTGTC 1500

ATCGGCGTGG CCGTCACCGT TTTGTATGCC TTTTGCACGC TCCTCCGCTG GAGGGACCCC 1560

GTCCGTGGCC AGAGCAGTGT GGGCGTGGCC GGAGTTCTGC TCATGTGCTT CAGTACCGCC 1620

GCCGGATTGG GATTGTCAGC CCTGCTCGGT ATCGTTTTCA ATGCGCTGAC CGCTGCCTAT 1680

GCGGAGAGCA ATCGGCGGGA GCAGACCAAG CTGATTCTCA AGAACGCCAG CACCCAGGTG 1740

GTTCCGTTTT TGGCCCTTGG TCTGGGCGTC GATCACATCT TCATAGTGGG ACCGAGCATC 1800

CTGTTCAGTG CCTGCAGCAC CGCAGGATCC TTCTTTGCGG CCGCCTTTAT TCCGGTGCCG 1860

GCTTTGAAGG TATTCTGTCT GCAGGCTGCC ATCGTAATGT GCTCCAATTT GGCAGCGGCT 1920

CTATTGGTTT TTCCGGCCAT GATTTCGTTG GATCTACGGA GACGTACCGC CGGCAGGGCG 1980

GACATCTTCT GCTGCTGTTT TCCGGTGTGG AAGGAACAGC CGAAGGTGGC ACCTCCGGTG 2040

CTGCCGCTGA ACAACAACAA CGGGCGCGGG GCCCGGCATC CGAAGAGCTG CAACAACAAC 2100

AGGGTGCCGC TGCCCGCCCA GAATCCTCTG CTGGAACAGA GGGCAGACAT CCCTGGGAGC 2160

AGTCACTCAC TGGCGTCCTT CTCCCTGGCA ACCTTCGCCT TTCAGCACTA CACTCCCTTC 2220

CTCATGCGCA GCTGGGTGAA GTTCCTGACC GTTATGGGTT TCCTGGCGGC CCTCATATCC 2280

AGCTTGTATG CCTCCACGCG CCTTCAGGAT GGCCTGGACA TTATTGATCT GGTGCCCAAG 2340

GACAGCAACG AGCACAAGTT CCTGGATGCT CAAACTCGGC TCTTTGGCTT CTACAGCATG 2400

TATGCGGTTA CCCAGGGCAA CTTTGAATAT CCCACCCAGC AGCAGTTGCT CAGGGACTAC 2460

CATGATTCCT TTGTGCGGGT GCCACATGTG ATCAAGAATG ATAACGGTGG ACTGCCGGAC 2520

TTCTGGCTGC TGCTCTTCAG CGAGTGGCTG GGTAATCTGC AAAAGATATT CGACGAGGAA 2580

TACCGCGACG GACGGCTGAC CAAGGAGTGC TGGTTCCCAA ACGCCAGCAG CGATGCCATC 2640

CTGGCCTACA AGCTAATCGT GCAAACCGGC CATGTGGACA ACCCCGTGGA CAAGGAACTG 2700

GTGCTCACCA ATCGCCTGGT CAACAGCGAT GGCATCATCA ACCAACGCGC CTTCTACAAC 2760

TATCTGTCGG CATGGGCCAC CAACGACGTC TTCGCCTACG GAGCTTCTCA GGGCAAATTG 2820

TATCCGGAAC CGCGCCAGTA TTTTCACCAA CCCAACGAGT ACGATCTTAA GATACCCAAG 2880

AGTCTGCCAT TGGTCTACGC TCAGATGCCC TTTTACCTCC ACGGACTAAC AGATACCTCG 2940

CAGATCAAGA CCCTGATAGG TCATATTCGC GACCTGAGCG TCAAGTACGA GGGCTTCGGC 3000

CTGCCCAACT ATCCATCGGG CATTCCCTTC ATCTTCTGGG AGCAGTACAT GACCCTGCGC 3060

TCCTCACTGG CCATGATCCT GGCCTGCGTG CTACTCGCCG CCCTGGTGCT GGTCTCCCTG 3120

CTCCTGCTCT CCGTTTGGGC CGCCGTTCTC GTGATCCTCA GCGTTCTGGC CTCGCTGGCC 3180

CAGATCTTTG GGGCCATGAC TCTGCTGGGC ATCAAACTCT CGGCCATTCC GGCAGTCATA 3240

CTCATCCTCA GCGTGGGCAT GATGCTGTGC TTCAATGTGC TGATATCACT GGGCTTCATG 3300

ACATCCGTTG GCAACCGACA GCGCCGCGTC CAGCTGAGCA TGCAGATGTC CCTGGGACCA 3360

CTTGTCCACG GCATGCTGAC CTCCGGAGTG GCCGTGTTCA TGCTCTCCAC GTCGCCCTTT 3420

GAGTTTGTGA TCCGGCACTT CTGCTGGCTT CTGCTGGTGG TCTTATGCGT TGGCGCCTGC 3480

AACAGCCTTT TGGTGTTCCC CATCCTACTG AGCATGGTGG GACCGGAGGC GGAGCTGGTG 3540

CCGCTGGAGC ATCCAGACCG CATATCCACG CCCTCTCCGC TGCCCGTGCG CAGCAGCAAG 3600

AGATCGGGCA AATCCTATGT GGTGCAGGGA TCGCGATCCT CGCGAGGCAG CTGCCAGAAG 3660

TCGCATCACC ACCACCACAA AGACCTTAAT GATCCATCGC TGACGACGAT CACCGAGGAG 3720

CCGCAGTCGT GGAAGTCCAG CAACTCGTCC ATCCAGATGC CCAATGATTG GACCTACCAG 3780

CCGCGGGAAC AGCGACCCGC CTCCTACGCG GCCCCGCCCC CCGCCTATCA CAAGGCCGCC 3840

GCCCAGCAGC ACCACCAGCA TCAGGGCCCG CCCACAACGC CCCCGCCTCC CTTCCCGACG 3900

GCCTATCCGC CGGAGCTGCA GAGCATCGTG GTGCAGCCGG AGGTGACGGT GGAGACGACG 3960

CACTCGGACA GCAACACCAC CAAGGTGACG GCCACGGCCA ACATCAAGGT GGAGCTGGCC 4020

ATGCCCGGCA GGGCGGTGCG CAGCTATAAC TTTACGAGTT AGCACTAGCA CTAGTTCCTG 4080

TAGCTATTAG GACGTATCTT TAGACTCTAG CCTAAGCCGT AACCCTATTT GTATCTGTAA 4140

AATCGATTTG TCCAGCGGGT CTGCTGAGGA TTTCGTTCTC ATGGATTCTC ATGGATTCTC 4200

ATGGATGCTT AAATGGCATG GTAATTGGCA AAATATCAAT TTTTGTGTCT CAAAAAGATG 4260

CATTAGCTTA TGGTTTCAAG ATACATTTTT AAAGAGTCCG CCAGATATTT ATATAAAAAA 4320

AATCCAAAAT CGACGTATCC ATGAAAATTG AAAAGCTAAG CAGACCCGTA TGTATGTATA 4380

TGTGTATGCA TGTTAGTTAA TTTCCCGAAG TCCGGTATTT ATAGCAGCTG CCTT 4434

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1285 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Arg Asp Ser Leu Pro Arg Val Pro Asp Thr His Gly Asp Val
1 5 10 15

Val Asp Glu Lys Leu Phe Ser Asp Leu Tyr Ile Arg Thr Ser Trp Val
20 25 30

Asp Ala Gln Val Ala Leu Asp Gln Ile Asp Lys Gly Lys Ala Arg Gly
35 40 45

Ser Arg Thr Ala Ile Tyr Leu Arg Ser Val Phe Gln Ser His Leu Glu
50 55 60

```
Thr Leu Gly Ser Ser Val Gln Lys His Ala Gly Lys Val Leu Phe Val
65                  70                  75                  80

Ala Ile Leu Val Leu Ser Thr Phe Cys Val Gly Leu Lys Ser Ala Gln
                85                  90                  95

Ile His Ser Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Arg Leu
            100                 105                 110

Glu Ala Glu Leu Ala Tyr Thr Gln Lys Thr Ile Gly Glu Asp Glu Ser
        115                 120                 125

Ala Thr His Gln Leu Leu Ile Gln Thr Thr His Asp Pro Asn Ala Ser
    130                 135                 140

Val Leu His Pro Gln Ala Leu Leu Ala His Leu Glu Val Leu Val Lys
145                 150                 155                 160

Ala Thr Ala Val Lys Val His Leu Tyr Asp Thr Glu Trp Gly Leu Arg
                165                 170                 175

Asp Met Cys Asn Met Pro Ser Thr Pro Ser Phe Glu Gly Ile Tyr Tyr
            180                 185                 190

Ile Glu Gln Ile Leu Arg His Leu Ile Pro Cys Ser Ile Ile Thr Pro
        195                 200                 205

Leu Asp Cys Phe Trp Glu Gly Ser Gln Leu Leu Gly Pro Glu Ser Ala
    210                 215                 220

Val Val Ile Pro Gly Leu Asn Gln Arg Leu Leu Trp Thr Thr Leu Asn
225                 230                 235                 240

Pro Ala Ser Val Met Gln Tyr Met Lys Gln Lys Met Ser Glu Glu Lys
                245                 250                 255

Ile Ser Phe Asp Phe Glu Thr Val Glu Gln Tyr Met Lys Arg Ala Ala
            260                 265                 270

Ile Gly Ser Gly Tyr Met Glu Lys Pro Cys Leu Asn Pro Leu Asn Pro
        275                 280                 285

Asn Cys Pro Asp Thr Ala Pro Asn Lys Asn Ser Thr Gln Pro Pro Asp
    290                 295                 300

Val Gly Ala Ile Leu Ser Gly Gly Cys Tyr Gly Tyr Ala Ala Lys His
305                 310                 315                 320

Met His Trp Pro Glu Glu Leu Ile Val Gly Gly Arg Lys Arg Asn Arg
                325                 330                 335

Ser Gly His Leu Arg Lys Ala Gln Ala Leu Gln Ser Val Val Gln Leu
            340                 345                 350

Met Thr Glu Lys Glu Met Tyr Asp Gln Trp Gln Asp Asn Tyr Lys Val
        355                 360                 365

His His Leu Gly Trp Thr Gln Glu Lys Ala Ala Glu Val Leu Asn Ala
    370                 375                 380

Trp Gln Arg Asn Phe Ser Arg Glu Val Glu Gln Leu Leu Arg Lys Gln
385                 390                 395                 400
```

Ser Arg Ile Ala Thr Asn Tyr Asp Ile Tyr Val Phe Ser Ser Ala Ala
405 410 415

Leu Asp Asp Ile Leu Ala Lys Phe Ser His Pro Ser Ala Leu Ser Ile
420 425 430

Val Ile Gly Val Ala Val Thr Val Leu Tyr Ala Phe Cys Thr Leu Leu
435 440 445

Arg Trp Arg Asp Pro Val Arg Gly Gln Ser Ser Val Gly Val Ala Gly
450 455 460

Val Leu Leu Met Cys Phe Ser Thr Ala Ala Gly Leu Gly Leu Ser Ala
465 470 475 480

Leu Leu Gly Ile Val Phe Asn Ala Leu Thr Ala Ala Tyr Ala Glu Ser
485 490 495

Asn Arg Arg Glu Gln Thr Lys Leu Ile Leu Lys Asn Ala Ser Thr Gln
500 505 510

Val Val Pro Phe Leu Ala Leu Gly Leu Gly Val Asp His Ile Phe Ile
515 520 525

Val Gly Pro Ser Ile Leu Phe Ser Ala Cys Ser Thr Ala Gly Ser Phe
530 535 540

Phe Ala Ala Ala Phe Ile Pro Val Pro Ala Leu Lys Val Phe Cys Leu
545 550 555 560

Gln Ala Ala Ile Val Met Cys Ser Asn Leu Ala Ala Ala Leu Leu Val
565 570 575

Phe Pro Ala Met Ile Ser Leu Asp Leu Arg Arg Arg Thr Ala Gly Arg
580 585 590

Ala Asp Ile Phe Cys Cys Cys Phe Pro Val Trp Lys Glu Gln Pro Lys
595 600 605

Val Ala Pro Pro Val Leu Pro Leu Asn Asn Asn Asn Gly Arg Gly Ala
610 615 620

Arg His Pro Lys Ser Cys Asn Asn Asn Arg Val Pro Leu Pro Ala Gln
625 630 635 640

Asn Pro Leu Leu Glu Gln Arg Ala Asp Ile Pro Gly Ser Ser His Ser
645 650 655

Leu Ala Ser Phe Ser Leu Ala Thr Phe Ala Phe Gln His Tyr Thr Pro
660 665 670

Phe Leu Met Arg Ser Trp Val Lys Phe Leu Thr Val Met Gly Phe Leu
675 680 685

Ala Ala Leu Ile Ser Ser Leu Tyr Ala Ser Thr Arg Leu Gln Asp Gly
690 695 700

Leu Asp Ile Ile Asp Leu Val Pro Lys Asp Ser Asn Glu His Lys Phe
705 710 715 720

Leu Asp Ala Gln Thr Arg Leu Phe Gly Phe Tyr Ser Met Tyr Ala Val
725 730 735

Thr Gln Gly Asn Phe Glu Tyr Pro Thr Gln Gln Gln Leu Leu Arg Asp

740 745 750

Tyr His Asp Ser Phe Arg Val Pro His Val Ile Lys Asn Asp Asn Gly
755 760 765

Gly Leu Pro Asp Phe Trp Leu Leu Leu Phe Ser Glu Trp Leu Gly Asn
770 775 780

Leu Gln Lys Ile Phe Asp Glu Glu Tyr Arg Asp Gly Arg Leu Thr Lys
785 790 795 800

Glu Cys Trp Phe Pro Asn Ala Ser Ser Asp Ala Ile Leu Ala Tyr Lys
805 810 815

Leu Ile Val Gln Thr Gly His Val Asp Asn Pro Val Asp Lys Glu Leu
820 825 830

Val Leu Thr Asn Arg Leu Val Asn Ser Asp Gly Ile Ile Asn Gln Arg
835 840 845

Ala Phe Tyr Asn Tyr Leu Ser Ala Trp Ala Thr Asn Asp Val Phe Ala
850 855 860

Tyr Gly Ala Ser Gln Gly Lys Leu Tyr Pro Glu Pro Arg Gln Tyr Phe
865 870 875 880

His Gln Pro Asn Glu Tyr Asp Leu Lys Ile Pro Lys Ser Leu Pro Leu
885 890 895

Val Tyr Ala Gln Met Pro Phe Tyr Leu His Gly Leu Thr Asp Thr Ser
900 905 910

Gln Ile Lys Thr Leu Ile Gly His Ile Arg Asp Leu Ser Val Lys Tyr
915 920 925

Glu Gly Phe Gly Leu Pro Asn Tyr Pro Ser Gly Ile Pro Phe Ile Phe
930 935 940

Trp Glu Gln Tyr Met Thr Leu Arg Ser Ser Leu Ala Met Ile Leu Ala
945 950 955 960

Cys Val Leu Leu Ala Ala Leu Val Leu Val Ser Leu Leu Leu Leu Ser
965 970 975

Val Trp Ala Ala Val Leu Val Ile Leu Ser Val Leu Ala Ser Leu Ala
980 985 990

Gln Ile Phe Gly Ala Met Thr Leu Leu Gly Ile Lys Leu Ser Ala Ile
995 1000 1005

Pro Ala Val Ile Leu Ile Leu Ser Val Gly Met Met Leu Cys Phe Asn
1010 1015 1020

Val Leu Ile Ser Leu Gly Phe Met Thr Ser Val Gly Asn Arg Gln Arg
1025 1030 1035 1040

Arg Val Gln Leu Ser Met Gln Met Ser Leu Gly Pro Leu Val His Gly
1045 1050 1055

Met Leu Thr Ser Gly Val Ala Val Phe Met Leu Ser Thr Ser Pro Phe
1060 1065 1070

Glu Phe Val Ile Arg His Phe Cys Trp Leu Leu Leu Val Val Leu Cys

1075 1080 1085

Val Gly Ala Cys Asn Ser Leu L u Val Phe Pro Ile Leu Leu Ser Met
1090 1095 1100

Val Gly Pro Glu Ala Glu Leu Val Pro Leu Glu His Pro Asp Arg Ile
1105 1110 1115 1120

Ser Thr Pro Ser Pro Leu Pro Val Arg Ser Ser Lys Arg Ser Gly Lys
1125 1130 1135

Ser Tyr Val Val Gln Gly Ser Arg Ser Ser Arg Gly Ser Cys Gln Lys
1140 1145 1150

Ser His His His His His Lys Asp Leu Asn Asp Pro Ser Leu Thr Thr
1155 1160 1165

Ile Thr Glu Glu Pro Gln Ser Trp Lys Ser Ser Asn Ser Ser Ile Gln
1170 1175 1180

Met Pro Asn Asp Trp Thr Tyr Gln Pro Arg Glu Gln Arg Pro Ala Ser
1185 1190 1195 1200

Tyr Ala Ala Pro Pro Pro Ala Tyr His Lys Ala Ala Ala Gln Gln His
1205 1210 1215

His Gln His Gln Gly Pro Pro Thr Thr Pro Pro Pro Pro Phe Pro Thr
1220 1225 1230

Ala Tyr Pro Pro Glu Leu Gln Ser Ile Val Val Gln Pro Glu Val Thr
1235 1240 1245

Val Glu Thr Thr His Ser Asp Ser Asn Thr Thr Lys Val Thr Ala Thr
1250 1255 1260

Ala Asn Ile Lys Val Glu Leu Ala Met Pro Gly Arg Ala Val Arg Ser
1265 1270 1275 1280

Tyr Asn Phe Thr Ser
1285

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 345 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAGGTCCATC AGCTTTGGAT ACAGGAAGGT GGTTCGCTCG AGCATGAGCT AGCCTACACG 60

CAGAAATCGC TCGGCGAGAT GGACTCCTCC ACGCACCAGC TGCTAATCCA AACNCCCAAA 120

GATATGGACG CCTCGATACT GCACCCGAAC GCGCTACTGA CGCACCTGGA CGTGGTGAAG 180

AAAGCGATCT CGGTGACGGT GCACATGTAC GACATCACGT GGAGNCTCAA GGACATGTGC 240

TACTCGCCCA GCATACCGAG NTTCGATACG CACTTTATCG AGCAGATCTT CGAGAACATC 300

ATACCGTGCG CGATCATCAC GCCGCTGGAT TGCTTTTGGG AGGGA 345

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Ser Leu Glu His Glu
1 5 10 15

Leu Ala Tyr Thr Gln Lys Ser Leu Gly Glu Met Asp Ser Ser Thr His
20 25 30

Gln Leu Leu Ile Gln Thr Pro Lys Asp Met Asp Ala Ser Ile Leu His
35 40 45

Pro Asn Ala Leu Leu Thr His Leu Asp Val Val Lys Lys Ala Ile Ser
50 55 60

Val Thr Val His Met Tyr Asp Ile Thr Trp Xaa Leu Lys Asp Met Cys
65 70 75 80

Tyr Ser Pro Ser Ile Pro Xaa Phe Asp Thr His Phe Ile Glu Gln Ile
85 90 95

Phe Glu Asn Ile Ile Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe
100 105 110

Trp Glu Gly
115

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5187 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC 60

CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCC 120

GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC 180

GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG 240

GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG 300

TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAAA AGAACTGCGG CAAGTTTTTG 360

GTTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG 420

ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT 480

ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA 540

AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGACTCAGCA 600

CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTTG 660

TGCTACAAAT CAGGGGAACT TATCACGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC 720

CTTTACCCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GGGAAGGGGC AAAGCTACAG 780

TGCGGGACAG CATACCTCCT AGGTAAGCCT CCTTTACGGT GGACAAACTT TGACCCCTTG 840

GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG 900

AATAAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA 960

GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT 1020

TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT 1080

GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAACTTGTCA GCGCTCACGC CCTGCAAACC 1140

ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC 1200

TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT 1260

TACGTGGAGG TGGTTCATCA AAGTGTCGCC CCAAACTCCA CTCAAAAGGT GCTTCCCTTC 1320

ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG 1380

GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC 1440

TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GGCGTCCTGT TGGTTGCGCT GTCAGTGGCT 1500

GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT 1560

TTGCCGTTTC TTGCTCTTGG TGTTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC 1620

AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGACTGGGGA GTGCCTCAAG 1680

CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTCATGGCC 1740

GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA 1800

TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA 1860

CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG 1920

ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCCA 1980

CCCCCATACA CCAGCCACAG CTTCGCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT 2040

CAGCTCCGCA CAGAGTATGA CCCTCACACG CACGTGTACT ACACCACCGC CGAGCCACGC 2100

TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC 2160

GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC 2220

CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT 2280

TTCCTCCTGA AACCCAAAGC CAAGGTTGTG GTAATCCTTC TTTTCCTGGG CTTGCTGGGG 2340

GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC 2400

CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC 2460

ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT 2520

CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA 2580

ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC 2640

TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCCTC 2700

GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG 2760

ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAATC CGAGCGCTTT CTACATCTAC 2820

CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG 2880

CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA 2940

ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA 3000

GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG 3060

AGCCTGGGAC TGTCCAGCTA CCCCAATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC 3120

AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTTCTAGTG 3180

TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG 3240

ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT 3300

GTGGTCATCC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG 3360

GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG 3420

TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG 3480

TCCGAATTTG ATTTCATTGT CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG 3540

GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCCT 3600

GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCACTC CTTCGCCTGA GCCGCCTCCA 3660

AGTGTCGTCC GGTTTGCCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC 3720

TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA 3780

GCACAGCAGG GTGCCGGAGG CCCTGCCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCCT 3840

GTCTTTGCCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT 3900

CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT 3960

CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCCCT ACAGACCGCG CAGAGACGCT 4020

TTTGAAATTT CTACTGAAGG GCATTCTGGC CCTAGCAATA GGGACCGCTC AGGGCCCCGT 4080

GGGGCCCGTT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC 4140

AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT 4200

CCCCCGCCTG GACCTGGGCG CAACCCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC 4260

CCTGAGACTG ATCACGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG 4320

AGGAGGGACT CAAAGGTGGA GGTCATAGAG CTACAGGACG TGGAATGTGA GGAGAGGCCG 4380

TGGGGGAGCA GCTCCAACTG AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA 4440

AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGGA ATTATGGGAA 4500

GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKKGTG AAATATTTCT ATAAATATTT 4560

AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTCC TGGGGCCTCT 4620

CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC 4680

TGTGCCACAA CCAAGCTTAA CTTAGTTTTA AAAAAAATCT CCCAGCATAT GTCGCTGCTG 4740

CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAAA TATTGCTTAT GTAATAGGAT 4800

TATTTGTAAA GGTTTCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860

ATGAATTGTT ACTGTTAACT TTTGAACACG CTATGCGTGG TAATTGTTTA ACGAGCAGAC 4920

ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTCGCATG 4980

GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040

TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100

GTGGGCTGGG AAGGTCCAGG TCTTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160

CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1434 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Ala Gly Asn Ala Ala Gly Ala Leu Gly Arg Gln Ala Gly
1 5 10 15

Gly Gly Arg Arg Arg Arg Thr Gly Gly Pro His Arg Ala Ala Pro Asp

```
            20                  25                  30

Arg Asp Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu
        35                  40                  45

Glu Gln Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp
    50                  55                  60

Leu Arg Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile
65                  70                  75                  80

Gln Lys Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly
                85                  90                  95

Ala Phe Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu
            100                 105                 110

Glu Leu Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr
        115                 120                 125
Thr Arg Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met
    130                 135                 140

Ile Gln Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala
145                 150                 155                 160

Leu Leu Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val
                165                 170                 175

Tyr Met Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser
            180                 185                 190

Gly Glu Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr
        195                 200                 205

Leu Tyr Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly
    210                 215                 220

Ala Lys Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu
225                 230                 235                 240

Arg Trp Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys
                245                 250                 255

Ile Asn Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu
            260                 265                 270

Val Gly His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro
        275                 280                 285

Asp Cys Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp
    290                 295                 300

Val Ala Leu Val Leu Asn Gly Gly Cys Gln Gly Leu Ser Arg Lys Tyr
305                 310                 315                 320

Met His Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ala
                325                 330                 335

Thr Gly Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu
            340                 345                 350

Met Thr Pro Lys Gln Met Tyr Glu His Phe Arg Gly Tyr Asp Tyr Val
        355                 360                 365
```

```
Ser His Ile Asn Trp Asn Glu Asp Arg Ala Ala Ala Ile Leu Glu Ala
    370                 375                 380

Trp Gln Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Pro Asn
385                 390                 395                 400

Ser Thr Gln Lys Val Leu Pro Phe Thr Thr Thr Thr Leu Asp Asp Ile
                405                 410                 415

Leu Lys Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr
            420                 425                 430

Leu Leu Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys
        435                 440                 445

Ser Lys Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala
    450                 455                 460

Leu Ser Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser
465                 470                 475                 480

Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val
                485                 490                 495

Gly Val Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly
            500                 505                 510

Gln Asn Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys
        515                 520                 525

Arg Thr Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala
    530                 535                 540

Phe Phe Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser
545                 550                 555                 560

Leu Gln Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu
                565                 570                 575

Ile Phe Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg
            580                 585                 590

Arg Leu Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val
        595                 600                 605

Ile Gln Val Glu Pro Gln Ala Tyr Thr Glu Pro His Ser Asn Thr Arg
    610                 615                 620

Tyr Ser Pro Pro Pro Pro Tyr Thr Ser His Ser Phe Ala His Glu Thr
625                 630                 635                 640

His Ile Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro
                645                 650                 655

His Thr His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser
            660                 665                 670

Val Gln Pro Val Thr Val Thr Gln Asp Asn Leu Ser Cys Gln Ser Pro
        675                 680                 685

Glu Ser Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser
    690                 695                 700
```

```
Ser Leu His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser
705                 710                 715                 720

Phe Ala Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys
                725                 730                 735

Val Val Val Ile Leu Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr
            740                 745                 750
Gly Thr Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro
        755                 760                 765

Arg Glu Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe
    770                 775                 780

Ser Phe Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn
785                 790                 795                 800

Ile Gln His Leu Leu Tyr Asp Leu His Lys Ser Phe Ser Asn Val Lys
                805                 810                 815

Tyr Val Met Leu Glu Glu Asn Lys Gln Leu Pro Gln Met Trp Leu His
            820                 825                 830

Tyr Phe Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp
        835                 840                 845

Trp Glu Thr Gly Arg Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp
    850                 855                 860

Asp Gly Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp
865                 870                 875                 880

Lys Pro Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala
                885                 890                 895

Asp Gly Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp
            900                 905                 910

Val Ser Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg
        915                 920                 925

Pro His Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu
    930                 935                 940

Thr Arg Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe
945                 950                 955                 960

Pro Phe Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala
                965                 970                 975

Ile Glu Lys Val Arg Val Ile Cys Asn Asn Tyr Thr Ser Leu Gly Leu
            980                 985                 990

Ser Ser Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile
        995                 1000                1005

Ser Leu Arg His Trp Leu Leu Leu Ser Ile Ser Val Val Leu Ala Cys
    1010                1015                1020

Thr Phe Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly
1025                1030                1035                1040
```

Ile Ile Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met
1045 1050 1055

Met Gly Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu
1060 1065 1070

Ile Ala Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu
1075 1080 1085

Ala Phe Leu Thr Ala Ile Gly Asp Lys Asn His Arg Ala Met Leu Ala
1090 1095 1100

Leu Glu His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu
1105 1110 1115 1120

Leu Gly Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg
1125 1130 1135

Tyr Phe Phe Ala Val Leu Ala Ile Leu Thr Val Leu Gly Val Leu Asn
1140 1145 1150

Gly Leu Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Cys Pro
1155 1160 1165

Glu Val Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro
1170 1175 1180

Glu Pro Pro Pro Ser Val Val Arg Phe Ala Val Pro Pro Gly His Thr
1185 1190 1195 1200

Asn Asn Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr
1205 1210 1215

Val Ser Gly Ile Ser Glu Glu Leu Arg Gln Tyr Glu Ala Gln Gln Gly
1220 1225 1230

Ala Gly Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro
1235 1240 1245

Val Phe Ala Arg Ser Thr Val Val His Pro Asp Ser Arg His Gln Pro
1250 1255 1260

Pro Leu Thr Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Ser
1265 1270 1275 1280

Pro Gly Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly
1285 1290 1295

Leu Arg Pro Pro Pro Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser
1300 1305 1310

Thr Glu Gly His Ser Gly Pro Ser Asn Arg Asp Arg Ser Gly Pro Arg
1315 1320 1325

Gly Ala Arg Ser His Asn Pro Arg Asn Pro Thr Ser Thr Ala Met Gly
1330 1335 1340

Ser Ser Val Pro Ser Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser
1345 1350 1355 1360

Ala Ser Val Thr Val Ala Val His Pro Pro Pro Gly Pro Gly Arg Asn
1365 1370 1375

Pro Arg Gly Gly Pro Cys Pro Gly Tyr Glu Ser Tyr Pro Glu Thr Asp
1380 1385 1390

His Gly Val Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu
1395 1400 1405

Arg Arg Asp Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys
1410 1415 1420

Glu Glu Arg Pro Trp Gly Ser Ser Ser Asn
1425 1430

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ile Val Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Phe Phe Trp Glu Gln Tyr

1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGACGAATTC AARGTNCAYC ARYTNTGG 28

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACGAATTC CYTCCCARAA RCANTC 26

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
GGACGAATTC YTNGANTGYT TYTGGGA 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATACCAGCC AAGCTTGTCN GGCCARTGCA T 31

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCCGGG GACCGCAAGG AGTGCCGCGG AAGCGCCCGA AGGACAGGCT CGCTCGGCGC 60

GCCGGCTCTC GCTCTTCCGC GAACTGGATG TGGGCAGCGG CGGCCGCAGA GACCTCGGGA 120

CCCCCGCGCA ATGTGGCAAT GGAAGGCGCA GGGTCTGACT CCCCGGCAGC GGCCGCGGCC 180

GCAGCGGCAG CAGCGCCCGC CGTGTGAGCA GCAGCAGCGG CTGGTCTGTC AACCGGAGCC 240

CGAGCCCGAG CAGCCTGCGG CCAGCAGCGT CCTCGCAAGC CGAGCGCCCA GGCGCGCCAG 300

GAGCCCGCAG CAGCGGCAGC AGCGCGCCGG GCCGCCCGGG AAGCCTCCGT CCCCGCGGCG 360

GCGGCGGCGG CGGCGGCGGC AACATGGCCT CGGCTGGTAA CGCCGCCGAG CCCCAGGACC 420

GCGGCGGCGG CGGCAGCGGC TGTATCGGTG CCCCGGGACG GCCGGCTGGA GGCGGGAGGC 480

GCAGACGGAC GGGGGGGCTG CGCCGTGCTG CCGCGCCGGA CCGGGACTAT CTGCACCGGC 540

CCAGCTACTG CGACGCCGCC TTCGCTCTGG AGCAGATTTC CAAGGGGAAG GCTACTGGCC 600

GGAAAGCGCC ACTGTGGCTG AGAGCGAAGT TTCAGAGACT CTTATTTAAA CTGGGTTGTT 660

ATATTCAAAA AAACTGCGGC AAGTTCTTGG TTGTGGGCCT CCTCATATTT GGGGCCTTCG 720

CGGTGGGATT AAAAGCAGCG AACCTCGAGA CCAACGTGGA GGAGCTGTGG GTGGAAGTTG 780

GAGGACGAGT AAGTCGTGAA TTAAATTATA CTCGCCAGAA GATTGGAGAA GAGGCTATGT 840

TTAATCCTCA ACTCATGATA CAGACCCCTA AAGAAGAAGG TGCTAATGTC CTGACCACAG 900

AAGCGCTCCT ACAACACCTG GACTCGGCAC TCCAGGCCAG CCGTGTCCAT GTATACATGT 960

ACAACAGGCA GTGGAAATTG GAACATTTGT GTTACAAATC AGGAGAGCTT ATCACAGAAA 1020

CAGGTTACAT GGATCAGATA ATAGAATATC TTTACCCTTG TTTGATTATT ACACCTTTGG 1080

ACTGCTTCTG GGAAGGGGCG AAATTACAGT CTGGGACAGC ATACCTCCTA GGTAAACCTC 1140

CTTTGCGGTG GACAAACTTC GACCCTTTGG AATTCCTGGA AGAGTTAAAG AAAATAAACT 1200

ATCAAGTGGA CAGCTGGGAG GAAATGCTGA ATAAGGCTGA GGTTGGTCAT GGTTACATGG 1260

ACCGCCCCTG CCTCAATCCG GCCGATCCAG ACTGCCCCGC CACAGCCCCC AACAAAAATT 1320

CAACCAAACC TCTTGATATG GCCCTTGTTT TGAATGGTGG ATGTCATGGC TTATCCAGAA 1380

AGTATATGCA CTGGCAGGAG GAGTTGATTG TGGGTGGCAC AGTCAAGAAC AGCACTGGAA 1440

AACTCGTCAG CGCCCATGCC CTGCAGACCA TGTTCCAGTT AATGACTCCC AAGCAAATGT 1500

ACGAGCACTT CAAGGGGTAC GAGTATGTCT CACACATCAA CTGGAACGAG GACAAAGCGG 1560

CAGCCATCCT GGAGGCCTGG CAGAGGACAT ATGTGGAGGT GGTTCATCAG AGTGTCGCAC 1620

AGAACTCCAC TCAAAAGGTG CTTTCCTTCA CCACCACGAC CCTGGACGAC ATCCTGAAAT 1680

CCTTCTCTGA CGTCAGTGTC ATCCGCGTGG CCAGCGGCTA CTTACTCATG CTCGCCTATG 1740

CCTGTCTAAC CATGCTGCGC TGGGACTGCT CCAAGTCCCA GGGTGCCGTG GGGCTGGCTG 1800

GCCTCCTGCT GGTTGCACTG TCAGTGGCTG CAGGACTGGG CCTGTGGTCA TTGATCGGAA 1860

TTTCCTTTAA CGCTGCAACA ACTCAGGTTT TGCCATTTCT CGCTCTTGGT GTTGGTGTGG 1920

ATGATGTTTT TCTTCTGGCC CACGCCTTCA GTGAAACAGG ACAGAATAAA AGAATCCCTT 1980

TTGAGGACAG GACCGGGGAG TGCCTGAAGC GCACAGGAGC CAGCGTGGCC CTCACGTCCA 2040

TCAGCAATGT CACAGCCTTC TTCATGGCCG CGTTAATCCC AATTCCCGCT CTGCGGGCGT 2100

TCTCCCTCCA GGCAGCGGTA GTAGTGGTGT TCAATTTTGC CATGGTTCTG CTCATTTTTC 2160

CTGCAATTCT CAGCATGGAT TTATATCGAC GCGAGGACAG GAGACTGGAT ATTTTCTGCT 2220

GTTTTACAAG CCCCTGCGTC AGCAGAGTGA TTCAGGTTGA ACCTCAGGCC TACACCGACA 2280

CACACGACAA TACCCGCTAC AGCCCCCCAC CTCCCTACAG CAGCCACAGC TTTGCCCATG 2340

AAACGCAGAT TACCATGCAG TCCACTGTCC AGCTCCGCAC GGAGTACGAC CCCCACACGC 2400

ACGTGTACTA CACCACCGCT GAGCCGCGCT CCGAGATCTC TGTGCAGCCC GTCACCGTGA 2460

CACAGGACAC CCTCAGCTGC CAGAGCCCAG AGAGCACCAG CTCCACAAGG GACCTGCTCT 2520

CCCAGTTCTC CGACTCCAGC CTCCACTGCC TCGAGCCCCC CTGTACGAAG TGGACACTCT 2580

CATCTTTTGC TGAGAAGCAC TATGCTCCTT TCCTCTTGAA ACCAAAAGCC AAGGTAGTGG 2640

TGATCTTCCT TTTTCTGGGC TTGCTGGGGG TCAGCCTTTA TGGCACCACC CGAGTGAGAG 2700

ACGGGCTGGA CCTTACGGAC ATTGTACCTC GGGAAACCAG AGAATATGAC TTTATTGCTG 2760

CACAATTCAA ATACTTTTCT TTCTACAACA TGTATATAGT CACCCAGAAA GCAGACTACC 2820

CGAATATCCA GCACTTACTT TACGACCTAC ACAGGAGTTT CAGTAACGTG AAGTATGTCA 2880

TGTTGGAAGA AAACAAACAG CTTCCCAAAA TGTGGCTGCA CTACTTCAGA GACTGGCTTC 2940

AGTCAGTTCA GGATGCATTT GACAGTGACT GGGAAACCGG GAAAATCATG CCAAACAATT 3000

ACAAGAATGG ATCAGACGAT GGAGTCCTTG CCTACAAACT CCTGGTGCAA ACCGGCAGCC 3060

GCGATAAGCC CATCGACATC AGCCAGTTGA CTAAACAGCG TCTGGTGGAT GCAGATGGCA 3120

TCATTAATCC CAGCGCTTTC TACATCTACC TGACGGCTTG GGTCAGCAAC GACCCCGTCG 3180

CGTATGCTGC CTCCCAGGCC AACATCCGGC CACACCGACC AGAATGGGTC CACGACAAAG 3240

CCGACTACAT GCCTGAAACA AGGCTGAGAA TCCCGGCAGC AGAGCCCATC GAGTATGCCC 3300

AGTTCCCTTT CTACCTCAAC GGGTTGCGGG ACACCTCAGA CTTTGTGGAG GCAATTGAAA 3360

AAGTAAGGAC CATCTGCAGC AACTATACGA GCCTGGGGCT GTCCAGTTAC CCCAACGGCT 3420

ACCCCTTCCT CTTCTGGGAG CAGTACATCG GCCTCCGCCA CTGGCTGCTG CTGTTCATCA 3480

GCGTGGTGTT GGCCTGCACA TTCCTCGTGT GCGCTGTCTT CCTTCTGAAC CCCTGGACGG 3540

CCGGGATCAT TGTGATGGTC CTGGCGCTGA TGACGGTCGA GCTGTTCGGC ATGATGGGCC 3600

TCATCGGAAT CAAGCTCAGT GCCGTGCCCG TGGTCATCCT GATCGCTTCT GTTGGCATAG 3660

GAGTGGAGTT CACCGTTCAC GTTGCTTTGG CCTTTCTGAC GGCCATCGGC GACAAGAACC 3720

GCAGGGCTGT GCTTGCCCTG GAGCACATGT TTGCACCCGT CCTGGATGGC GCCGTGTCCA 3780

CTCTGCTGGG AGTGCTGATG CTGGCGGGAT CTGAGTTCGA CTTCATTGTC AGGTATTTCT 3840

TTGCTGTGCT GGCGATCCTC ACCATCCTCG GCGTTCTCAA TGGGCTGGTT TTGCTTCCCG 3900

TGCTTTTGTC TTTCTTTGGA CCATATCCTG AGGTGTCTCC AGCCAACGGC TTGAACCGCC 3960

TGCCCACACC CTCCCCTGAG CCACCCCCCA GCGTGGTCCG CTTCGCCATG CCGCCCGGCC 4020

ACACGCACAG CGGGTCTGAT TCCTCCGACT CGGAGTATAG TTCCCAGACG ACAGTGTCAG 4080

GCCTCAGCGA GGAGCTTCGG CACTACGAGG CCCAGCAGGG CGCGGGAGGC CCTGCCCACC 4140

AAGTGATCGT GGAAGCCACA GAAAACCCCG TCTTCGCCCA CTCCACTGTG GTCCATCCCG 4200

AATCCAGGCA TCACCCACCC TCGAACCCGA GACAGCAGCC CCACCTGGAC TCAGGGTCCC 4260

TGCCTCCCGG ACGGCAAGGC CAGCAGCCCC GCAGGGACCC CCCCAGAGAA GGCTTGTGGC 4320

CACCCCTCTA CAGACCGCGC AGAGACGCTT TTGAAATTTC TACTGAAGGG CATTCTGGCC 4380

CTAGCAATAG GGCCCGCTGG GGCCCTCGCG GGGCCCGTTC TCACAACCCT CGGAACCCAG 4440

CGTCCACTGC CATGGGCAGC TCCGTGCCCG GCTACTGCCA GCCCATCACC ACTGTGACGG 4500

CTTCTGCCTC CGTGACTGTC GCCGTGCACC CGCCGCCTGT CCCTGGGCCT GGGCGGAACC 4560

CCCGAGGGGG ACTCTGCCCA GGCTACCCTG AGACTGACCA CGGCCTGTTT GAGGACCCCC 4620

ACGTGCCTTT CCACGTCCGG TGTGAGAGGA GGGATTCGAA GGTGGAAGTC ATTGAGCTGC 4680

AGGACGTGGA ATGCGAGGAG AGGCCCCGGG GAAGCAGCTC CAACTGAGGG TGATTAAAAT 4740

CTGAAGCAAA GAGGCCAAAG ATTGGAAACC CCCCACCCCC ACCTCTTTCC AGAACTGCTT 4800

GAAGAGAACT GGTTGGAGTT ATGGAAAAGA TGCCCTGTGC CAGGACAGCA GTTCATTGTT 4860

ACTGTAACCG ATTGTATTAT TTTGTTAAAT ATTTCTATAA ATATTTAAGA GATGTACACA 4920

TGTGTAATAT AGGAAGGAAG GATGTAAAGT GGTATGATCT GGGGCTTCTC CACTCCTGCC 4980

CCAGAGTGTG GAGGCCACAG TGGGGCCTCT CCGTATTTGT GCATTGGGCT CCGTGCCACA 5040

ACCAAGCTTC ATTAGTCTTA AATTTCAGCA TATGTTGCTG CTGCTTAAAT ATTGTATAAT 5100

TTACTTGTAT AATTCTATGC AAATATTGCT TATGTAATAG GATTATTTTG TAAAGGTTTC 5160

TGTTTAAAAT ATTTTAAATT TGCATATCAC AACCCTGTGG TAGTATGAAA TGTTACTGTT 5220

AACTTTCAAA CACGCTATGC GTGATAATTT TTTTGTTTAA TGAGCAGATA TGAAGAAAGC 5280

CCGGAATT 5288

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1447 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ala Ser Ala Gly Asn Ala Ala Glu Pro Gln Asp Arg Gly Gly Gly
1 5 10 15

Gly Ser Gly Cys Ile Gly Ala Pro Gly Arg Pro Ala Gly Gly Gly Arg
20 25 30

Arg Arg Arg Thr Gly Gly Leu Arg Arg Ala Ala Ala Pro Asp Arg Asp
35 40 45

Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu Glu Gln
50 55 60

Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp Leu Arg
65 70 75 80

Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile Gln Lys
85 90 95

Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly Ala Phe
100 105 110

Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu Glu Leu
115 120 125

Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr Thr Arg
130 135 140

Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met Ile Gln
145 150 155 160

Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala Leu Leu
165 170 175

Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val Tyr Met

180 185 190

Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser Gly Glu
195 200 205

Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr Leu Tyr
210 215 220

Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ala Lys
225 230 235 240

Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu Arg Trp
245 250 255

Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys Ile Asn
260 265 270

Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu Val Gly
275 280 285

His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro Asp Cys
290 295 300

Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp Met Ala
305 310 315 320

Leu Val Leu Asn Gly Gly Cys His Gly Leu Ser Arg Lys Tyr Met His
325 330 335

Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ser Thr Gly
340 345 350

Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu Met Thr
355 360 365

Pro Lys Gln Met Tyr Glu His Phe Lys Gly Tyr Glu Tyr Val Ser His
370 375 380

Ile Asn Trp Asn Glu Asp Lys Ala Ala Ala Ile Leu Glu Ala Trp Gln
385 390 395 400

Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Gln Asn Ser Thr
405 410 415

Gln Lys Val Leu Ser Phe Thr Thr Thr Thr Leu Asp Asp Ile Leu Lys
420 425 430

Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr Leu Leu
435 440 445

Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys Ser Lys
450 455 460

Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala Leu Ser
465 470 475 480

Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser Phe Asn
485 490 495

Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val Gly Val
500 505 510

Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly Gln Asn

515 520 525

Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys Arg Thr
530 535 540

Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala Phe Phe
545 550 555 560

Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser Leu Gln
565 570 575

Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu Ile Phe
580 585 590

Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg Arg Leu
595 600 605

Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val Ile Gln
610 615 620

Val Glu Pro Gln Ala Tyr Thr Asp Thr His Asp Asn Thr Arg Tyr Ser
625 630 635 640

Pro Pro Pro Pro Tyr Ser Ser His Ser Phe Ala His Glu Thr Gln Ile
645 650 655

Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro His Thr
660 665 670

His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser Val Gln
675 680 685

Pro Val Thr Val Thr Gln Asp Thr Leu Ser Cys Gln Ser Pro Glu Ser
690 695 700

Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser Ser Leu
705 710 715 720

His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser Phe Ala
725 730 735

Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys Val Val
740 745 750

Val Ile Phe Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr Gly Thr
755 760 765

Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro Arg Glu
770 775 780

Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe Ser Phe
785 790 795 800

Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn Ile Gln
805 810 815

His Leu Leu Tyr Asp Leu His Arg Ser Phe Ser Asn Val Lys Tyr Val
820 825 830

Met Leu Glu Glu Asn Lys Gln Leu Pro Lys Met Trp Leu His Tyr Phe
835 840 845

Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp Trp Glu
850 855 860

Thr Gly Lys Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp Asp Gly
865 870 875 880

Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp Lys Pro
885 890 895

Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala Asp Gly
900 905 910

Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp Val Ser
915 920 925

Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg Pro His
930 935 940

Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu Thr Arg
945 950 955 960

Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe Pro Phe
965 970 975

Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala Ile Glu
980 985 990

Lys Val Arg Thr Ile Cys Ser Asn Tyr Thr Ser Leu Gly Leu Ser Ser
995 1000 1005

Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile Gly Leu
1010 1015 1020

Arg His Trp Leu Leu Leu Phe Ile Ser Val Val Leu Ala Cys Thr Phe
1025 1030 1035 1040

Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly Ile Ile
1045 1050 1055

Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met Met Gly
1060 1065 1070

Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu Ile Ala
1075 1080 1085

Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu Ala Phe
1090 1095 1100

Leu Thr Ala Ile Gly Asp Lys Asn Arg Arg Ala Val Leu Ala Leu Glu
1105 1110 1115 1120

His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu Leu Gly
1125 1130 1135

Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg Tyr Phe
1140 1145 1150

Phe Ala Val Leu Ala Ile Leu Thr Ile Leu Gly Val Leu Asn Gly Leu
1155 1160 1165

Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Tyr Pro Glu Val
1170 1175 1180

Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro Glu Pro
1185 1190 1195 1200

```
Pro Pro Ser Val Val Arg Phe Ala Met Pro Pro Gly His Thr His Ser
                1205                1210                1215

Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr Val Ser
            1220                1225                1230

Gly Leu Ser Glu Glu Leu Arg His Tyr Glu Ala Gln Gln Gly Ala Gly
        1235                1240                1245

Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro Val Phe
    1250                1255                1260

Ala His Ser Thr Val Val His Pro Glu Ser Arg His His Pro Pro Ser
1265                1270                1275                1280

Asn Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Pro Pro Gly
                1285                1290                1295

Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly Leu Trp
            1300                1305                1310

Pro Pro Leu Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser Thr Glu
        1315                1320                1325

Gly His Ser Gly Pro Ser Asn Arg Ala Arg Trp Gly Pro Arg Gly Ala
    1330                1335                1340

Arg Ser His Asn Pro Arg Asn Pro Ala Ser Thr Ala Met Gly Ser Ser
1345                1350                1355                1360

Val Pro Gly Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser Ala Ser
                1365                1370                1375

Val Thr Val Ala Val His Pro Pro Pro Val Pro Gly Pro Gly Arg Asn
            1380                1385                1390

Pro Arg Gly Gly Leu Cys Pro Gly Tyr Pro Glu Thr Asp His Gly Leu
        1395                1400                1405

Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu Arg Arg Asp
    1410                1415                1420

Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys Glu Glu Arg
1425                1430                1435                1440

Pro Arg Gly Ser Ser Ser Asn
                1445
```

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a *patched* protein other than *Drosophila melanogaster patched* protein, or fragment of at least about 12 nt in length thereof, as other than an intact chromosome.

2. An isolated nucleic acid according to Claim 1 wherein said *patched* protein is mosquito, butterfly or beetle.

3. An isolated nucleic acid according to Claim 1, wherein said patched protein is a mammalian protein.

4. An isolated nucleic acid according to Claim 3, wherein said *patched* protein is human.

5. In isolated nucleic acid according to Claim 3, wherein said patched protein is mouse.

6. An expression cassette comprising a transcriptional initiation region functional in an expression host, a nucleic acid having a sequence of o the isolated nucleic acid according to Claim 1 under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said expression host.

7. A cell comprising an expression cassette according to Claim 6 as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host cell and the cellular progeny of said host cell.

8. A method for producing patched protein, said method comprising growing a cell according to Claim 7, whereby said *patched* protein is expressed; and isolating said patched protein free of other proteins.

9. A purified polypeptide composition comprising at least 50 weight % of the protein present as a patched protein or a fragment thereof, other than *Drosophila melanogaster patched* protein.

10. A purified polypeptide composition according to Claim 9, wherein said patched protein is a mammalian protein.

11. A purified polypeptide composition according to Claim 10, wherein said patched protein is human.

12. A purified polypeptide composition according to Claim 10, wherein said patched protein is mouse.

13. A monoclonal antibody binding specifically to a patched protein other than *Drosophila melanogaster patched* protein.

14. A method for diagnosing a genetic predisposition for at least one of developmental abnormalities and cancer in an individual, the method comprising:

— detecting the presence of a predisposing mutation in a *patched* gene in the germline of said individual,

— wherein the presence of said predisposing mutation indicates that said individual has a genetic predisposition for at least one of developmental abnormalities and

cancer.

15. A method according to Claim 14, wherein said genetic predisposition is basal cell nevus syndrome.

16. A method according to Claim 14, wherein said detecting step comprises analyzing the DNA of said individual.

17. A method according to Claim 14, wherein said detecting step comprises functional analysis of patched protein function.

18. A method according to Claim 14, wherein said detecting step comprisesdetecting antibody binding to abnormal patched protein.

19. A method for characterizing the phenotype of a tumor, the method comprising:

— detecting the presence of an oncogenic patched mutation in said tumor, wherein the presence of said oncogenic mutation indicates that said tumor has a patched-associated phenotype.

20. A method according to Claim 19, wherein said tumor is a carcinoma.

21. A method according to Claim 20, wherein said carcinoma is a basal cell carcinoma.

22. A method according to Claim 19, wherein said detecting step comprises analyzing the DNA of said tumor.

23. A method according to Claim 19, wherein said detecting step comprises functional analysis of patched protein function.

24. A method according to Claim 19, wherein said detecting step comprises detecting antibody binding to abnormal patched protein.

25. A genetically engineered mammalian cell predisposed to develop basal cell carcinoma as a result of transfection of said mammalian cell with at least one DNA construct comprising an altered patched *or hedgehog* gene.

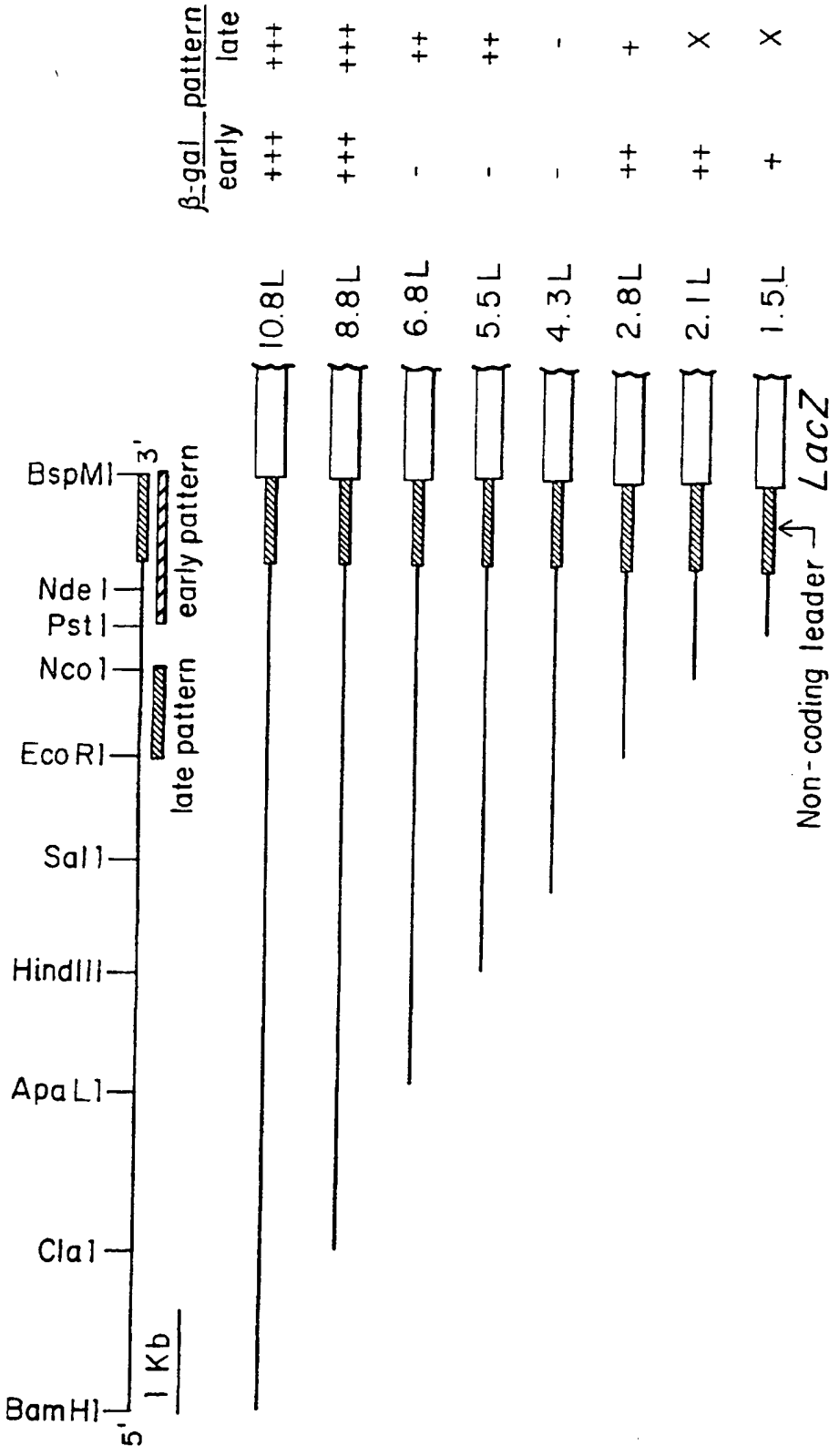
β-gal pattern
early late
10.8L +++ +++
8.8L +++ +++
6.8L - ++
5.5L - ++
4.3L - -
2.8L ++ +
2.1L ++ X
1.5L + X
BspMI
Nde I
Pst I
Nco I
Eco RI
Sal I
HindIII
Apa LI
Cla I
BamHI
5'
3'
1 Kb
early pattern
late pattern
Non-coding leader
LacZ

FIG. 1

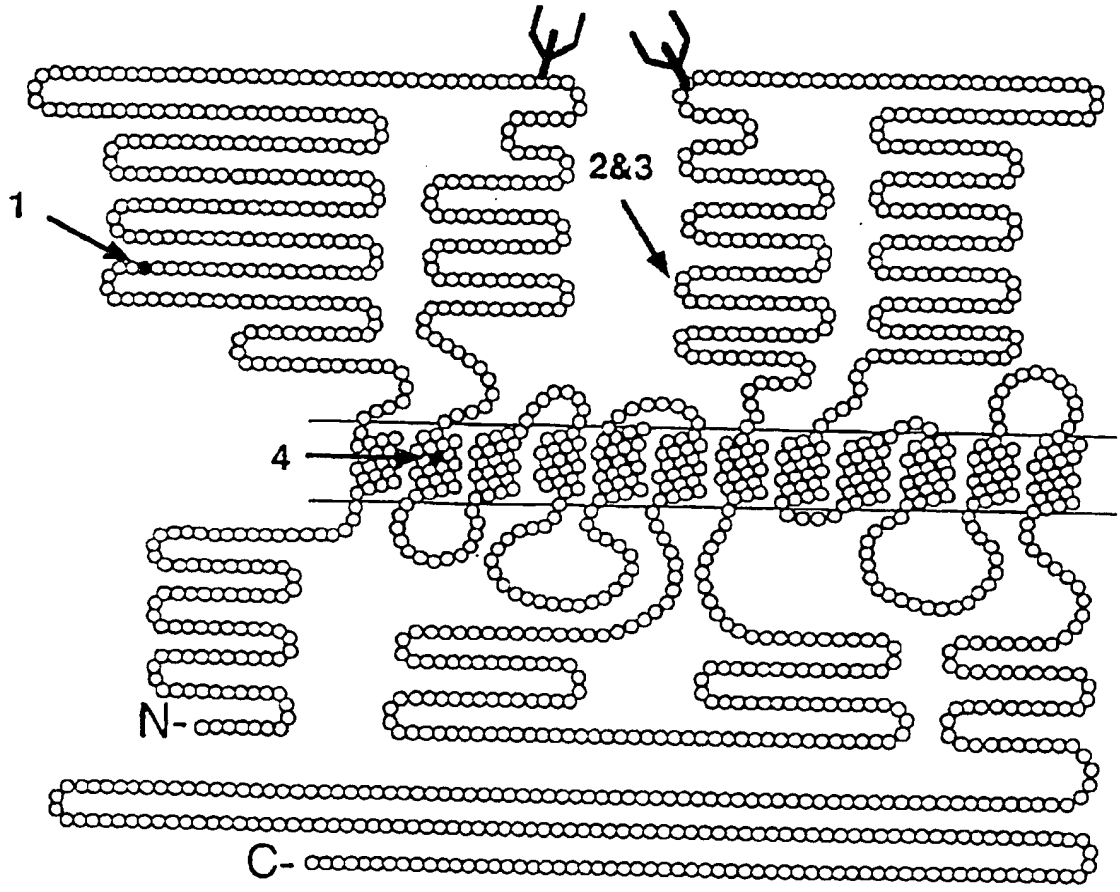

FIG. 2